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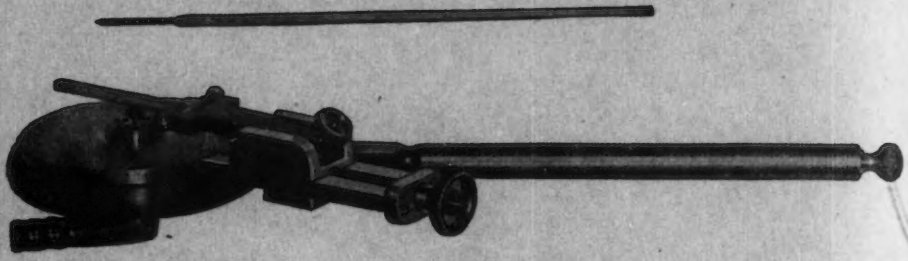

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PHYSIOLOGICAL VARIATIONS OF THE CARDIAC OUTPUT IN MAN

X. The Effect of Variations in the Environmental Temperature on the Pulse Rate, Blood Pressure, Oxygen Comsumption, Arterio-Venous Oxygen Difference, and Cardiac Output of Normal Individuals

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Received for publication June 28, 1930

That changes in the external temperature of the environment affect the cardiovascular system of man is manifested by the obvious vascular changes occurring in the skin, by the marked increase in pulse rate at high temperatures, etc. Our knowledge of the exact nature of these vascular changes or their quantitative relationships is, however, quite uncertain and incomplete. A study of the cardiac output at various environmental temperatures should help to elucidate this problem. Previous attempts to apply this function, however, have been unsatisfactory (due to the gross errors involved in the experimental methods employed) and have led to contradictory conclusions. The earlier work on this subject by Kraus (1910), Bornstein, (1911, 1913), Schapals (1912), and Lindhard (1915) has been reviewed and criticized by Tigerstedt (1918). More recent work by Barcroft and Marshall (1923) and Henderson and Haggard (1925) is open to similar criticisms due to the inaccurate methods employed for determining the cardiac output. It is worthy of note that relatively small errors in cardiac output determinations (as evidenced by the work cited above) render worthless even qualitative deductions regarding circulatory changes. This fact has not been appreciated by many recent workers who, despite a tacit appreciation of the doubtful value of the methods which they have employed, have nevertheless persisted in drawing far-reaching conclusions, under the false assumption that though in error as to the absolute values, their results are valid insofar as comparative conclusions are concerned.

Experimental methods employed. The present determinations were

carried out by the methods and with the precautions described in previous papers of this series (Grollman, 1929, 1930). The cardiac output was determined by the use of acetylene, blood pressure by the Tycos recording sphygmomanometer, oxygen consumption by the Krogh spirometer, and pulse rate by radial palpation. The experiments were conducted in an insolated room the temperature of which could be varied and maintained constant to within 0.5° by a system of coolers (circulating brine) and electric heaters. An electric fan circulated the air to maintain a uniform temperature, but was not allowed to blow on the subject of the experiment in order to avoid sensory stimulation which might affect the pulse rate (Lyth, 1911) or other vascular phenomena. The humidity of the chamber was main-

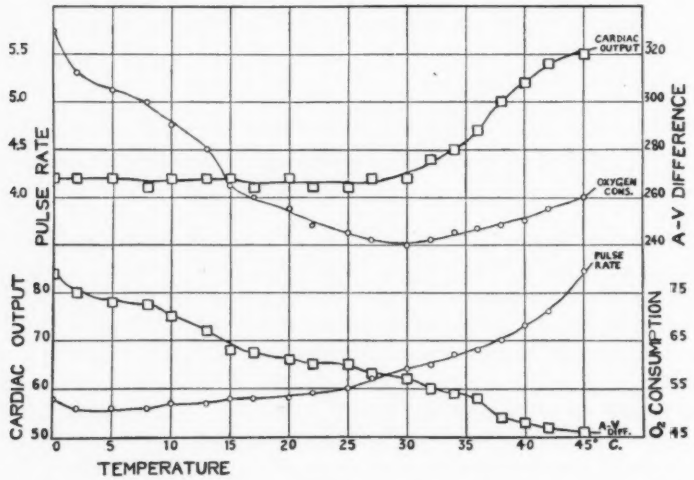

Fig. 1. The cardiac output (liters per minute), pulse rate (per minute), arteriovenous oxygen difference (cc. per liter), and oxygen consumption (cc. per minute) of a normal individual over the temperature range of 0° to 45° centigrade.

tained relatively low in order to avoid any discomfort sufficient to affect the functions measured through psychic disturbances (Grollman, 1929b).

The subjects during the experiment were dressed in their usual attire and the results quoted in this paper refer to that condition. The effect of the various temperatures on the nude body were also studied, the result at any given temperature being (as might be anticipated) that obtained at a somewhat lower temperature when the clothing was retained.

Because of the great number of experiments necessary to cover the temperature range investigated (0° to 45°), it was deemed undesirable to conduct a complete series on any individual on a single day. The temperature

range from 0° to room temperature was usually investigated on one day and from room temperature to 45° on another. All the experiments were conducted in the basal condition, and hence the various series of determinations, although performed on different days, are comparable (Grollman, 1930a), and may be grouped together as a single series.

RESULTS. The effects of changes in the environmental temperature on the various functions studied during the present investigation are shown in tables 1 and 2, and are graphically represented in figure 1.

In an experiment at any given temperature, duplicate determinations, which agreed within the limits of the experimental errors, were made, and the averages are recorded in the present paper. At least 20 minutes elapsed before conducting an experiment at any given temperature in order to allow the subject to adjust himself to that temperature. As shown in table 1, the cardiovascular reactions to temperature changes are extremely rapid in their development and the same results are obtained at any period from 10 minutes to an hour after subjecting the subject to a given temperature. Table 1 demonstrates the results obtained at 0°, 20°, and 40°.

A total of five subjects was studied during the present investigation. The results obtained on the different individuals were uniform both as regards the general deductions to be drawn and as regards the order of magnitude of the changes observed. Consequently typical results on only one subject need be recorded in the present paper.

Pulse and systolic output. The pulse rate changes only slightly over the temperature range of 0 to 20°, varying from 56 at 2° to 58 at 20°. With further elevations in temperature, however, the pulse rate rises with greater rapidity, increasing 6 beats in the temperature interval of 20° to 30°, 9 beats between 30° and 40°, and 11 beats per minute between 40° and 45°. Increasing temperature thus exerts a progressively greater effect on the pulse rate at elevated temperatures. This change in pulse rate follows the change in cardiac output rather closely resulting in a fairly constant stroke-volume or systolic output per beat. The latter function is shown in the last column of table 2 where it gradually decreases from a maximum of 75 at 2° to a minimum of 66 at 30° after which it again rises to 71 at 42°, but decreases again to 65 at 45°.

Blood pressure. The systolic blood pressure gradually declines (table 2) from 116 at 0° to 100 at 32° where it remains, to rise slightly again at 45°. The diastolic pressure undergoes a similar change, the pulse pressure thus remaining practically constant. This relative constancy of the pulse pressure indicates that the observed changes in cardiac output are being accompanied by concomitant changes in the vascular bed so that with increasing temperature the heart responds in pumping an additional amount of blood equal to that which now is required to pass through areas previously constricted, *viz.*, the skin.

TABLE 1

The metabolic and vascular functions of a normal individual subjected for varying periods of time to several environmental temperatures

ROOM TEMPERATURE	DURATION OF EXPOSURE	PULSE RATE	BLOOD PRESSURE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT
°C.	minutes	per minute	mm. Hg	cc. per minute	cc. per liter	liters per minute
0	10	56	115/74	320	76	4.2
	30	56	110/69	310	75	4.1
	60	58	116/75	330	79	4.2
20	10	58	105/64	255	61	4.2
	30	57	105/65	250	60	4.2
	60	58	106/65	250	59	4.2
40	10	75	100/60	248	48	5.0
	30	76	100/60	250	47	5.3
	60	77	102/62	255	50	5.1

TABLE 2

The effect of the environmental temperature on the pulse rate, blood pressure, oxygen consumption, arterio-venous oxygen difference, cardiac output, and systolic output per beat of a normal human subject

ROOM TEMPERATURE	PULSE RATE	BLOOD PRESSURE	OXYGEN CONSUMPTION	ARTERIO-VENOUS OXYGEN DIFFERENCE	CARDIAC OUTPUT	SYSTOLIC OUTPUT
°C.	per minute	mm. Hg	cc. per minute	cc. per liter	liters per minute	cc. per heart beat
0	58	116/70	330	79	4.2	72
2	56	115/74	312	75	4.2	75
5	56	110/70	305	73	4.2	75
8	56	109/68	300	73	4.1	73
10	57	108/67	290	70	4.2	74
13	57	108/67	280	67	4.2	74
15	58	108/65	265	63	4.2	72
17	58	107/65	260	63	4.1	71
20	58	106/62	255	61	4.2	72
22	59	106/62	248	60	4.1	70
25	60	104/62	245	60	4.1	68
27	62	102/60	242	58	4.2	68
30	64	101/62	240	57	4.2	66
32	65	100/60	242	55	4.4	68
34	67	100/60	245	54	4.5	67
36	68	100/58	247	53	4.7	70
38	70	100/58	248	49	5.0	71
40	73	100/60	250	48	5.2	71
42	76	100/62	255	47	5.4	71
45	84	104/62	260	46	5.5	65

The blood pressure of man is usually considered as a rather labile and variable function. Thus the clinically accepted limits of normalcy cover a wide range. This current conception of a great variability in the blood pressure has not been substantiated in the large series of determinations which have been carried out during the course of the investigations on the cardiac output under various physiological conditions reported in this series of communications. The reason for the extraordinary constancy of blood pressure thus encountered is, no doubt, to be attributed to the maintenance of strictly basal (as evidenced by their minimal values) and constant (as evidenced by their negligible variations from day to day) conditions. It would, therefore, seem logical to exact the same strict requirements in studying blood pressure changes as is done in metabolic and certain other physiological functions. The failure to do this has, no doubt, rendered invalid or inconclusive the deductions of many studies in this field.

Not only have the basal blood pressure values been found to be extraordinarily constant but various physiological conditions (with the notable exception of muscular exercise and psychic disturbance) produce, in general, relatively slight (10 per cent) changes in the blood pressure. There is thus a tendency on the part of the organism to maintain this function relatively constant as compared with the more variable cardiac output and the very labile pulse rate.

Oxygen consumption. The oxygen consumption, as is well known, is markedly affected by temperature changes of the environment, increasing rapidly with decreasing temperatures below 20°. The cause of this rise has been the subject of much controversy (Lusk, 1928). Voit (1878) and Rubner (1890) considered the increased metabolism to be a manifestation of a chemical regulation on the part of the organism. Loewy (1890), Johansson (1897) and Sjöström (1913), on the other hand, attributed the rise in oxygen consumption to the additional metabolism due to shivering. Even where muscular movements are not evident, one must consider the increased muscular activity due to an increase in tone of the general musculature at lower temperatures. Cannon, Querido, Britton, and Bright (1926-27), in support of the views of Voit and Rubner, attribute to epinephrin a calorigenic property which, in conjunction with the coarse adjustment by shivering, maintains a constant body temperature with decreasing environmental temperatures.

With temperatures above 20° one finds a gradual but slight decline in the oxygen consumption till a minimum is reached at 30°, after which it again slowly rises with increasing temperature. The gradual decline over the temperature range of 20 to 30° is probably due to a diminution in tone of the musculature and greater relaxation with increasing temperature.

The oxygen consumption considered as a measure of the chemical proc-

esses occurring in the cells of the body will be subject to change by two general factors. The first of these factors acts by altering the intensity of the chemical activity of the cells or the number of cells in activity at any time (e.g., glandular activity or muscular exercise). The second general factor will act by changing the internal environment of the cells. The effect of drugs, ingestion of water, and the like are examples of the second type of factors which would be expected to affect the metabolism as measured by the oxygen consumption.

As regards the effect of temperature on the oxygen consumption both of the above general factors might conceivably be active. The increased muscular activity (as manifested in shivering or increased muscular tone) will obviously produce marked changes in oxygen consumption. The change in body temperature, although slight, will, however, also by changing the reaction velocity of the processes occurring in the cells, produce changes in the oxygen consumption. At low temperatures, the decrease in oxygen consumption thus produced will be masked by the much greater increases produced by muscular activity, enhanced by the effect of epinephrine, as described by Cannon and his collaborators. The increase in oxygen consumption (20 cubic centimeters per minute) which was observed when the temperature was increased from 30° to 45° (table 1), may well, however, be due in part to the increased reaction velocity of the chemical processes occurring in the cells due to the slight but definite increase in body temperature. Besides this factor, the observed increase is in part to be attributed to the increased metabolism of the sweat glands, respiratory mechanism, and heart due to the increased activity which these structures assume at high temperatures.

Cardiac output. The results of table 2 and figure 1 show that the cardiac output is practically constant over the temperature range of 0° to room temperature and is only affected by temperatures above 30°. The observed changes in cardiac output differ markedly from what has generally been accepted as occurring at low temperatures. The increase in cardiac output at high temperatures is generally admitted to be a manifestation of the extra blood which circulates through the skin in response to heat. It has, however, also been supposed that cold produces an opposite effect, *viz.*, a decrease in cardiac output due to constriction of the peripheral vessels. As seen in table 2 and figure 1, however, this view is erroneous. The cardiac output remains practically constant over the temperature range of 0° to 25°. Cooling below room temperature does not decrease the cardiac output, any diminution in the blood flow through the skin being negligible or compensated for by a corresponding increase elsewhere, probably through the somatic musculature or viscera.

Arterio-venous oxygen difference. The arterio-venous oxygen difference gradually decreases from its maximum value, 79, at an environmental

temperature of 0° to a minimum of 46 at 45°. The gradual increase with decreasing temperatures below 20° is consistent with the results obtained in a study of muscular exercise (Grollman, unpublished observations) in which it was found that increasing oxygen consumption (due to muscular exercise) is at times accompanied by an increase in the arterio-venous oxygen difference so as to maintain a constant cardiac output. The effect of heat is to gradually decrease the arterio-venous oxygen difference from 60 at room temperature to 46 at 45°. This change is readily appreciated when we consider the relatively great increase in blood flow, accompanying this change in temperature, through an area (the skin) whose metabolic activity is relatively small compared to its blood supply.

Discussion. The changes in the cardiac output observed when the environmental temperature varied from 0° to 45° may be considered as a manifestation of two processes. Between 0° and room temperature one is dealing, for the most part, with a condition of light exercise and since such exercise is without much effect on the cardiac output (as shall be shown in a subsequent paper of this series), one would not expect a change in cardiac output on reducing the temperature of the surroundings. The increase in cardiac output on elevating the temperature from room temperature to 45° amounts to about 1.3 liters for an individual of average size. This quantity will vary, of course, with different individuals depending chiefly on their surface area and the vascularity of their skin. In the subjects studied during the present investigation it varied only 0.2 liter from the value quoted above. The subject of the experiment quoted in table 2 had a surface area of 1.8 square meters. The increase in the volume of blood flowing through the skin (with an external temperature of 45°) is, therefore, approximately 700 cubic centimeters per minute. Meakins (1919-20), Barcroft and Nagahashi (1921), and Goldschmidt and Light (1925) have determined the variations in the oxygen content of the blood from a superficial vein after immersing the hand in a water bath at various temperatures. Barcroft and Nagahashi, from their data, calculate the blood flow through the skin to be about fifty times as great when the arm is in a hot bath as when it is subjected to cold. Applying the same method of calculation to Meakins' data gives an approximately similar result.

The fact that no change in cardiac output was observed over the temperature range 0° to 28° is not inconsistent with the marked changes in the degree of saturation of the venous blood from a superficial area, as observed by the above mentioned workers. It must be remembered that a ten-fold change in the volume of blood traversing the skin of a given area would represent an increase in blood flow of only a small fraction of a liter, a change not detectable in measurements of the total blood flow through the body. It is quite probable that there is a regular change in the blood flow through the skin as one changes the environmental temperature from 0° to 45°.

In the range of 28 to 45° this change amounting to about 1.3 liters is readily detectable while the change from 0° to 28° is probably much less than 100 cc. since the blood flow through the skin having already been greatly reduced cannot be decreased much further without producing an absolute ischemia. This view is in accord with plethysmographic measurements as observed by Hewlett (1911).

Any decrease in blood flow through the skin at temperatures from 0° to 25° must, as stated above, be interpreted as being small or compensated for by a corresponding increase in the flow through other tissues. If the view of Cannon and his collaborators (1926–7) concerning the calorigenic function of epinephrin be true, one would expect this substance to increase the blood flow as has been demonstrated by Euler and Liljestrand (1929) and as occurs in psychic disturbances (Grollman, 1929b). This increase in the blood flow due to the action of epinephrin may be considered as compensating for the decrease due to the constriction in the skin vessels, thus resulting in the constant total blood flow which was observed. In any case the quantity of blood thus diverted from the skin must be small and the absence of any increase in the total blood flow at low temperatures must be taken as evidence against the view that epinephrine plays the predominant part in producing the increased oxygen consumptions observed at low temperatures. Increased muscular activity (by obvious shivering or by increasing muscular tone) must, therefore, be considered as the predominant factor in maintaining the body temperature, the calorigenic property of epinephrine playing a minor rôle. A similar argument may be applied to the changes in pulse rate which were observed.

The numerous experiments carried out on animals to determine the effect of temperature on the circulation (Tigerstedt, 1918, 1922; Uyeno, 1923, and others) show a great variability in their results and conclusions. Unfortunately most of these experiments have been carried out on anesthetized and operated animals and hence the results are not comparable to those obtained on the normal organism. One might expect a difference in the reaction of the cardio-vascular system of a furred animal as compared to man due to the difference in vascularity and the distribution of sweat glands in these animals. Moreover, one must consider the possible effect of the more marked hyperpnea which occurs—particularly in the dog—at high temperatures. The hyperpnea in man at a temperature of 45° is insufficient to appreciably affect the cardiac output as has been previously demonstrated (Grollman, 1930b). This temperature may suffice, however, to appreciably affect the cardiac output of other animals. Despite the above differences, which might quantitatively render the results on the lower animals different from those obtained on man, one would expect the same general changes to occur in the normal unanesthetized animal. The only experimental data on such animals are those of Marshall (1926) who, in

two dogs, found the cardiac output to increase from 2.71 and 1.36 liters to 5.31 and 3.66 liters, respectively, after subjecting the animals to a cold bath. The great increase in oxygen consumption and pulse which accompanied this change in cardiac output and the high normal pulse rates (70 to 83) of the animals in the resting condition makes it possible that the observed increases are in great part due to an excessive muscular activity and psychic disturbance rather than to a direct action of cold. This difficulty in maintaining perfect basal conditions and in avoiding variations other than those being investigated renders the lower animals less suitable as subjects for the study of cardio-vascular phenomena than man. Moreover, the accuracy and reproducibility of the results obtainable by the methods which are applicable at present to man, exceed that obtainable by direct puncture of the heart, in the lower animals.

It is conceivable, however, that the response of man to cold is quite different from that of the dog and other lower animals. Thus, Cannon and his collaborators (1926–27) observed marked increases in the pulse rate of denervated cats subjected to low temperatures which they attribute to the action of epinephrine liberated in response to the cold. It is quite possible that this calorigenic action of epinephrine (although playing a minor rôle in the case of man, as discussed above) may play the predominant rôle in increasing the metabolism of the cat or dog. The marked increases in pulse rate and cardiac output of the dog in response to cold as observed by Marshall (1926), would thus be expected to occur if we assume that epinephrine (or some other chemical regulator which acts on both the cardio-vascular and metabolic functions) plays a greater part in these animals than it does in man.

The effect of hot and cold baths on the cardiovascular system has been investigated by a number of observers because of the clinical interest attached to such studies. Due to the greater conductivity of water, as compared to air, the temperature effect of a water bath at any given temperature on the circulation will differ markedly from the results obtained when air at the same temperature is used as the surrounding medium. This is to be expected on the basis of purely physical considerations and has been de nonstrated by Goldschmidt and Light (1925). One has, moreover, to consider the possible sensory effect of the water particularly when it is in motion (Rubner, 1903; Lyth, 1911) or when carbon dioxide is circulated through it. A final factor differentiating a water from an air bath is the possible hydrostatic effect of the column of water in compressing the extrathoracic veins. This factor has been demonstrated by Tigerstedt (1918) to be effective in the rabbit but it is doubtful whether in man (where the upright posture has led to the development of a more stable condition) such a factor has any appreciable effect on the circulation.

It may be pointed out that the views expressed in the present paper, be-

ing based upon experiments on the normal subject, are not transferable to pathological cases. Indeed, it seems very likely, in view of the paradoxical blanching of the skin or so-called "inverse vascular effect" of heat which has been observed in certain pathological conditions (Schmidt, 1923; Kauffmann, 1924; Lange, 1925; and others), that the vascular adjustments of such subjects to environmental temperature changes are quite different from the normal. Other clinical observations on the effect of temperature on the cardiovascular manifestations in disease render a study of the effect of temperature on the cardiac output and its related functions in pathological conditions desirable.

SUMMARY

A study was made of the cardiovascular and metabolic changes occurring in the normal human subject over a temperature range of 0° to 45° centigrade. The pulse rate rises progressively with increasing temperature, the changes below room temperature being slight. The blood pressure undergoes a change of only about 10 per cent throughout this range with only slight variations in the pulse pressure. The metabolism declines with increasing temperature, to a minimum, at about 30°, after which it again rises slowly. The nature of these changes in the metabolism and their probable causation is discussed. The cardiac output is constant over the temperature range of 0° to about 28°. At temperatures over 28° the cardiac output gradually increases with increasing temperature giving at 45° a value (for the average sized individual) 1.3 liters above the value at normal room temperature. These effects of temperature on the cardiac output are explained as being the resultants of two diverse phenomena. The constancy of the cardiac output with moderate degrees of cold is analogous to its constancy with slight muscular activity. The increase at higher temperatures represents the peripheral dilatation occurring under these conditions. The effect of baths (as compared to air as the environmental medium) and the possible differences to be encountered in pathological cases are also discussed. The bearing of the present results and those obtained on other animals on the theory of the calorigenic function of epinephrin is indicated.

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PHYSIOLOGICAL VARIATIONS IN THE CARDIAC OUTPUT OF MAN

XI. The Pulse Rate, Blood Pressure, Oxygen Consumption, Arterio-Venous Oxygen Difference, and Cardiac Output of Man During Normal Nocturnal Sleep

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As part of the general study of the cardiac output during various physiological conditions, of which this paper is one of a series, an investigation of the cardio-vascular system during sleep was undertaken. Data concerning the physiological functions during sleep are desirable to aid in the elucidation of this as yet unsatisfactorily explained state. Although the theories based on the "vasoconstriction" origin of sleep have been practically discarded in recent years, the view that marked changes occur in the circulatory system during sleep is still held. A determination of the cardiac output during this state would throw light on the rôle which such changes play in the organism. Heretofore, all studies of the cardio-vascular system during sleep have been limited chiefly to pulse, blood pressure, or plethysmographic studies, no attempt having been made to determine the cardiac output during sleep or during the waking state at night.

In the present investigation an answer has been sought to the two following problems:

1. Is the sleeping state (as opposed to the waking state at the same time of the night and under the same conditions of complete rest) characterized by any peculiarities in the pulse rate, blood pressure, oxygen consumption, arterio-venous oxygen difference, or cardiac output?

2. How do these functions vary during the course of the night?

To answer these two questions determinations were made during sleep and compared with those obtained a few moments later after awakening the subject. Such determinations were continued at intervals throughout the course of the night in the sleeping and waking states. A direct comparison of the value of a given physiological function thus derived while the subject is asleep with one made shortly afterwards when the subject has been awakened should allow one to conclude regarding the effect of sleep *per se* on the function measured. This procedure has seldom been

utilized in the numerous researches dealing with sleep, the observers usually being content to compare the values obtained during sleep with those obtained during the preceding day while the subject was awake. To attribute an observed decrease in the value of a given function obtained in this way to sleep rather than to the prolonged physical rest incidental to the sleeping state is fallacious and has given rise to many current erroneous conceptions. To consider such changes as causal factors in the production of sleep is but another stage of this fallacy.

EXPERIMENTAL METHODS EMPLOYED. Although it is obviously difficult to secure the coöperation of a subject in any experimental procedure while he is asleep, this does not render a determination of the cardiac output impossible. The arterio-venous oxygen difference derived in the determination of the cardiac output refers to the condition of the mixed venous blood before the beginning of the rebreathing procedure. Hence, if one awakens the subject suddenly and if the latter completes the rebreathing procedure within the time of a single circulation of the blood, the arterio-venous oxygen difference thus obtained will refer to the subject's condition before his awakening. The result thus obtained divided into the oxygen consumption determined during sleep will give the cardiac output during this condition.

Acetylene and the general procedures used in the course of the studies described in this series were utilized during the present investigation. The acetylene mixture with the attached sampling tubes, evacuated in the usual manner, were arranged on a single stand which could be carried quietly and quickly into the room in which the subject was sleeping. All such preparations were made in an adjoining room to avoid any noise which might affect the subject's sleep. The experimenter awakened the sleeper, adjusted the nose-clip, and completed the determination in the usual manner. After some practice, it was possible for the subject to begin the rebreathing within 3 seconds and complete the entire procedure in 20 to 23 seconds after his awakening. The results thus obtained, as mentioned above, will refer to the sleeping state. To compare these values with those obtained under the same conditions in the waking state, a second determination was made (the subject having in the meantime remained awake but completely relaxed) 15 minutes later.

The pulse rates were determined by recording graphically the pulsations from the brachial artery by means of a blood pressure cuff connected to a tambour. The pressure in the cuff was raised to about 70 mm. of Hg when a determination was desired. The objection to such measurements is that the sensory stimulation on applying pressure to the arm possibly affects the depth of sleep of the subject. The agreement of the present results with those obtained by electro-cardiographic or auscultatory methods (Klewitz, 1913, 1919; Boas and Weiss, 1929) speaks against the validity of this objection.

Blood pressure determinations were made by the use of the Tycos recording sphygmomanometer. The same objection to these measurements may be raised as was mentioned in the preceding paragraph concerning the pulse rate determinations. The objection in the case of the blood pressure determinations is, indeed, more pertinent, since the necessity of applying a higher pressure often resulted in awakening the subject, thus indicating the possibility of affecting the sleep even in cases where no awakening occurred. In view, however, of the results on the cardiac output which shall be discussed later, and in which the objection raised against the pulse rate or the blood pressure determinations is not applicable, the results obtained refer, it is believed, to the true sleeping state and have not been markedly affected by the procedure.

The oxygen consumption during sleep was determined by the use of the helmet, devised by Prof. F. G. Benedict (unpublished work), which was demonstrated at the Nutrition Laboratory of the Carnegie Institution during the XIIIth International Physiological Congress. Attempts to use various forms of facial masks invariably failed. After accustoming himself to the helmet, the subject readily fell asleep. Determinations of the oxygen consumption over short intervals of time were then alternately made in the sleeping and waking states.

The subjects of the present investigation were trained in the technique of the procedures used and were accustomed to the laboratory surroundings where the experiments were performed. These are important requirements in all experiments in which comparison is made of results during sleeping and waking states. Any slight disturbance on the part of the subject, such as annoyance at being awakened, might easily result in obtaining higher values in the waking than in the sleeping state. All lights and extraneous noises were excluded, except during an actual determination of the cardiac output. Under the conditions of the experiment, except when aroused, the subject was able to sleep as long and as deeply as was his usual custom at home. When awakened for any experiment, the subject continued in the state of physical relaxation characteristic of sleep and soon fell asleep again when left to himself.

Results. To avoid awakening the subject too frequently during a single night, it was necessary to assemble the data obtained on any one subject during several nights, and such composite results are recorded in the accompanying table. The results for the cardiac outputs and oxygen consumptions recorded in table 1 for the waking state were obtained 15 minutes after the value for the sleeping state, at the same hour, was determined. In the case of the pulse rate and blood pressure readings only a few minutes elapsed between the determinations in the sleeping and waking states at any given hour.

The subject of the experiments ate his evening meal at about 5 p.m.

and refrained from further food or drink (other than minimal quantities of water necessary to quench thirst) during the evening. He retired at about 10 p.m. after the usual day's activities and was asleep within half an hour after retiring. No drugs were used to induce sleep.

Three subjects were studied during the present investigation. The same conclusions are to be drawn from the data obtained on all subjects and hence the results on only one of them are recorded in the present paper. Table 1 gives the typical results illustrative of the deductions which may be drawn from the present work.

TABLE 1

The variations in the pulse rate, blood pressure, oxygen consumption, arterio-venous oxygen difference, and cardiac output throughout the night, asleep and awake

TIME	PULSE RATE PER MINUTE		BLOOD PRESSURE		OXYGEN CONSUMPTION		ARTERIO-VENOUS OXYGEN DIFFERENCE		CARDIAC OUTPUT	
	Asleep	Awake	Asleep	Awake	Asleep	Awake	Asleep	Awake	Asleep	Awake
			mm. Hg	*mm. Hg*	*cc. per minute*	*cc. per minute*	*cc. per liter*	*cc. per liter*	*liters per minute*	*liters per minute*
10:00 p.m.		70		108/70		280		60		4.7
10:30 to 11 p.m.	64	70	103/66	105/70	270	270	61	60	4.4	4.5
11:30 to 12 p.m.	61	66	100/68	98/67	246	251	65	64	3.8	4.0
12:30 to 1 a.m.	58	62	98/66	98/67	246	245	60	58	4.1	4.2
1:30 to 2 a.m.	52	60	96/65	97/65	240	239	63	64	3.8	3.7
2:30 to 3 a.m.	51	58	95/65	95/65	240	238	65	67	3.7	3.6
3:30 to 4 a.m.	50	57	92/61	93/65	230	236	67	66	3.4	3.6
4:30 to 5 a.m.	53	55	95/59	98/62	225	230	66	68	3.4	3.4
5:30 to 6 a.m.	53	56	100/65	102/67	216	220	64	62	3.4	3.5
6:30 to 7 a.m.	55	58	100/67	100/67	230	232	63	65	3.7	3.8
Average*.....	55	60	98/65	98/66	238	240	64	64	3.7	3.8

* The average does not include the measurements obtained at 10 p.m. before the subject fell asleep.

Pulse rate. As seen in table 1, the pulse rate at any given hour is markedly lower during sleep than when the subject is awake. The pulse rate per minute during the night was, on an average, 5 beats lower when the subject was asleep than when he was awake. The average pulse rate for this subject in bed soon after awaking was about 60, whereas its value at 4 a.m. in the sleeping state was only 50. There is an actual slowing of the heart rate during sleep below that encountered during the waking hours in the most relaxed state of physical rest.

The pulse rate, as given in table 1, both for the sleeping and waking states is seen to reach a minimum at about 4 a.m. after which it again rises slightly until the subject's awakening. This minimum was observed

about a century ago by Gruetzmann (1831) but has been overlooked by most subsequent observers.

Blood pressure. The blood pressure during sleep has been the subject of numerous studies but the conclusions of different observers conflict markedly. Many[1] (Howell, 1897; Brush and Fayerweather, 1901; Müller, 1921, *et alii*) claim that the blood pressure is actually lower during sleep than in the waking state. Close scrutiny of the experimental results quoted does not convince one of the validity of this conclusion. Many of the experiments were done on patients suffering from cardiovascular disease, while others were performed after the administration of soporifics. Many observers have compared the results obtained during sleep with those obtained during the preceding evening, no account being taken of the drop in blood pressure which would have resulted from the long rest, independent of sleep. Hill (1898) first demonstrated that the drop in blood pressure observed during sleep was not greater than that attained by resting in bed while awake for an equal period of time. He ascribed the low blood pressure observed during sleep to the complete physical rest and relaxation of the subject but the paucity of his experimental data has led subsequent workers to overlook his conclusions. Pierón (1913) cites an experiment which is in accord with Hill's conclusion.

The present experiments (table 1) confirm Hill's and Pierón's views and refute the idea that the blood pressure is lower during sleep than when the subject is awake. That is, *there is no specific change in the blood pressure which is associated with the sleeping state,* but the observed values occur irrespective of whether the subject is asleep or awake, so long as he is completely at rest. This is shown in the comparison of the values of the fourth and fifth columns of table 1. It will be noted that the blood pressure gradually drops to a minimum at about 4 a.m. (synchronous with the minimum in the pulse rate) and rises again to the basal resting value which is observed when the subject awakes in the morning. As in the case of the pulse rate, the blood pressure drops about 8 mm. below the basal values of the morning several hours before awaking.

The blood pressure values during sleep are of the same order of magnitude as those observed by Müller (1921), Blankenhorn and Campbell (1925), Landis (1925), and others. The marked rises on awakening observed by Katsch and Pansdorf (1922) in hospital patients are to be attributed to secondary effects of excitement incidental to the awakening. Thus Landis (1925) also observed that the blood pressure might rise from a sleep level of 94/68 to 110/64 if the subject be suddenly awakened. The

[1] In view of the numerous reviews on the sleep problem no attempt is made in this paper to cite all extant publications dealing with the subjects discussed. Such references may be obtained in the reviews of Piéron (1913), Ebbecke (1926), Miculicich (1928), and Kleitman (1929).

complete lack of excitatory factors in the present work explains the avoidance of these secondary rises in blood pressure on awakening.

The attainment of minimum values of the pulse and blood pressure several hours before awakening, as observed in the present work, was also demonstrated by Blankenhorn and Campbell (1925).

Cardiac output. As seen in table 1, the arterio-venous oxygen difference is not markedly affected by the sleeping state. It increases during the night over its value during the day (from 60 to 67), the maximum values occurring usually during periods of profound sleep. The cardiac output (columns 10 and 11 of table 1) similarly drops very rapidly to a minimum value at about 5 a.m. The values of the cardiac output during sleep are not lower than those obtained under the same conditions but with the subject awake, so that we must conclude that sleep *per se* is not associated with any diminution in activity of the cardio-vascular system (except as regards the pulse rate change described above). However, the minimum cardiac output observed is less than the resting basal value obtained in the morning. Thus, as in the case of the pulse rate, blood pressure, and oxygen consumption, the cardiac output reaches a *sub-basal*[2] value during the course of the night.

Oxygen consumption. As seen in columns 6 and 7 of table 1, the oxygen consumption is practically the same when the subject is awake as when he is asleep, *i.e., the sleeping state is not characterized by any peculiar diminution in the metabolism as compared to the waking state at the same hour of the night.* The oxygen consumption decreases rapidly in the early hours of the night to a minimum early in the morning which is about 10 per cent lower than the basal value as observed in the morning after awaking.

The identity (within the experimental error) of the values for the oxygen consumption at any given hour during sleep and when the subject is awake, agrees with the findings of Loewy (1891), Johansson (1898), and Delcourt-Bernard and Mayer (1925) who came to the same conclusion. However, the *sub-basal* values obtained between 3 and 6 a.m. (table 1) explain the apparently contradictory conclusion of Benedict (1915) who in calorimetric studies demonstrated a consistently lower metabolism of his fasting subject when asleep during the night than when he was awake during the early morning. The diminution during sleep as observed on Benedict's subject is greater than that calculated from the results of figure 1, but considering the difference in experimental conditions, qualitative agreement is all that is to be expected. We must thus conclude that although the metabolism during sleep is the same as it is when awake with complete physical relaxation at the same hour of the night, it is actually

[2] The term *sub-basal* is used in this paper to denote values of any given function which are lower than those obtained in the morning in the recumbent position, under the conditions under which the so-called basal values are obtainable.

sub-basal during the early hours of the morning (probably throughout the greater part of the night if the subject is in the fasting state as was the case of Benedict's subject).

Variations of the observed physiological functions with the nature of the sleep. The values for the various functions studied, as given in table 1, were found to be easily modified by the state of the subject. The subject upon whom the values in table 1 were obtained slept peacefully and deeply throughout the night except when aroused for experimental studies. When the sleep was not peaceful but was marked by restlessness results differing markedly from those of table 1 were encountered. Thus during a night in which the subject, upon whom the determinations quoted in table 1 were made, was disturbed by nightmares and dreams (due probably to an indiscretion of diet on the preceding day) the pulse rate was 70 at 1 a.m., 74 at 3 a.m., and 75 at 6 a.m.; the blood pressure 106/68, 112/73, and 110/67 at the same times, values considerably above those obtained in restful slumber.[3] The oxygen consumption, however, was not elevated above the usual values. During a night in which the subject slept well but in which the room temperature was about 29°, all of the functions studied were definitely elevated to an extent which would be anticipated from the effects of temperature, as described in the preceding paper of this series (Grollman, 1930a). At any given time of the night, however, the general relation between the values of any given function when the subject was asleep and when he was awake, showed the same relation to one another as the values recorded in table 1.

Kohlschütter (1869), Mönninghoff and Piesbergen (1883), and numerous subsequent observers have demonstrated the cyclic nature of the intensity of sleep throughout the night. Mönninghoff and Piesbergen found that the maximum intensity of sleep occurs at about $1\frac{3}{4}$ hours after retiring and then gradually decreases until waking. Subsequent work has demonstrated that although this condition obtains in many subjects it is not the universal rule (Ebbecke, 1926). The question arises as to the relation of the time of occurrence of the change in the functions as given in table 1 to the above mentioned intensity curve of sleep. The subject of table 1 showed a sleep-curve (as determined roughly by the ease with which he

[3] The values quoted are much lower than those which would be anticipated from the work of MacWilliam (1923) who claims that the blood pressure may undergo very marked rises during troubled dreams. MacWilliam's determinations were not made during the course of the dreams but soon after awakening. The objection, therefore, may be raised (as has been done by Kleitman (1929)) as to whether the increases noted by MacWilliam really occur during sleep or whether they are the results of an excitement aroused in the subject when he regains consciousness on awakening and is temporarily terrified by his recent dream. The fact that the blood pressure may rise rapidly on awakening is evident from the published curves of Katsch and Pansdorf (1922) and the results of Landis (1925).

was awakened) which was similar to that described by Mönninghoff and Piesbergen, *i.e.*, the period of his maximum intensity of sleep occurred at about 1 a.m. The minimum in the values of the various functions studied occurred, on the other hand, as we have seen, some hours later. Moreover, this same gradual decline over a period of from 4 to 6 hours after retiring occurred in the other subjects investigated irrespective of their sleep curve. This independence of the time in which minimal values for the pulse rate, blood pressure, *etc.*, are obtained to the maximum intensity of sleep, is readily explicable. As is well known, the pulse rate, blood pressure, oxygen consumption, and, no doubt, other functions when elevated above their basal values, *e.g.*, by exercise, resume their pristine values only slowly. On prolonged relaxation they progressively decrease in magnitude with time. Hence we would expect them to drop gradually during the course of the night unless sleep could effect their decrease. The results of table 1 have demonstrated the independence of the functions to the state of sleep, *per se*, and we would expect, therefore, the attainment of their minimal values to be dependent on time, as was actually observed, rather than on the depth of sleep.

It might, however, be further expected that the minimal values should be attained at the very end of the resting period, *i.e.*, at 7 a.m., instead of before this period, as was observed. This apparent inconsistency is explained, however, if we remember that the *sub-basal* values are probably the resultants of an extreme degree of physical relaxation concomitant with deep sleep. In the later hours of the sleeping period, this relaxation is less complete and hence we find a slight increase over the minimal values obtained several hours previously. One might also explain the attainment of these *sub-basal* values as resultants, or at least as concomitants, of a diurnal rhythm leading also to the minimum body temperature observed at the same hour of the morning.

Discussion. The general deductions to be drawn from the results of the present study are: 1. In so far as the blood pressure, oxygen consumption, arterio-venous oxygen difference, and cardiac output of normal individuals are concerned, sleep, as contrasted to the waking state, at the same hour and under the same conditions of physical rest, is not characterized by any appreciable diminution in the magnitude of these functions. 2. The pulse rate is definitely lower in the sleeping than in the waking state. 3. There is a gradual decline in the magnitudes of all of the functions studied which undergo a progressive decrease to *sub-basal* values during the night.

It may, at first thought, appear strange that the pulse rate should manifest such definite variations during sleep as compared to the other cardiovascular functions. This is not surprising, however, when one appreciates the true significance of the pulse rate in its relation to changes in the

cardio-vascular system. The pulse rate, as has previously been demonstrated in several papers of this series, is the most labile of all the cardio-vascular functions. Its changes are in no wise related, necessarily, to changes in cardiac output, for example, but the pulse must be considered as a purely nervous mechanism whose variations are not always reflected in other vascular changes. The pulse rate (being under a rapidly adjustable nervous control) is easily affected while other functions such as blood pressure or cardiac output, being less labile, require more profound changes for their alteration. It is very probable that the acoustic and optic stimuli which inevitably occur in the waking state are sufficient to account for the difference in pulse rate observed in sleep as compared to the waking state. Such nervous stimuli are insufficient, however, to affect the blood pressure, cardiac output, or oxygen consumption.

The cardiac output during sleep may be considered as representing the minimum requirement of the organism consistent with an adequate blood supply to the various tissues. It is usually stated that there is an abeyance of the activity of the bodily organs during sleep,[4] according to which view one might expect a far greater decrease in cardiac output during sleep than is actually observed. Such a view, however, is based on a comparison with the conditions during daily activity rather than during the basal post-absorptive state. Except for the lacrymal, salivary, and rhino-laryngeal secretions, which are thought to be reduced during sleep (Ebbecke, 1926), there is no evidence for the reduction in activity of other glands or organs other than that which might be expected from the absence of digestive and muscular activity. The usual diminution in urine volume during the night is explicable on the basis of lesser activity and on the lack of ingestion of fluids during this time. Thus Simpson (1926), in 17 experiments in which the subject slept during the day, could demonstrate a decrease in urinary volume in only one instance.[5]

The cardiac output during the night is thus that which obtains during conditions of absolute rest, in the recumbent basal condition, except for the period from 3 to 6 a.m. (in the experiment of table 1) when its value is 6 per cent lower than the basal value. This decrease corresponds in

[4] The demonstration of an apparent change in the resistance of the body (which may be considered as evidence of the occurrence of profound somatic changes) during sleep as described by Waller (1919), Farmer and Chambers (1924–25), and Richter (1926), has been refuted by Landis (1927) who has indicated the errors involved in the fundamental assumptions underlying the methods used by the above-mentioned investigators.

[5] The recent paper of Zitowitsch, demonstrating a periodicity and diurnal rhythm in the rate of diuresis in the dog may appear to be in conflict with the conception brought forward here. However, such periodicity in kidney function may well be a concomitant of a parallel periodicity as regards activity in the dog and not related specifically to renal activity.

magnitude to the *sub-basal* value attained by the oxygen consumption and other functions recorded in table 1 as well as the drop in body temperature[6] which is known to occur at this period. One might consider this decrease in the values of these various functions as a manifestation of a general decline in the activity of the general protoplasmic processes or as resultants of the attainment of complete relaxation and rest of the various organs of the body.

The fact that the decreases in cardiac output, blood pressure, and oxygen consumption during sleep are of the same order of magnitude and such as would be expected to occur in complete rest, renders improbable the view that sleep is attended by any marked vasomotor changes, redistribution of blood, cerebral anemia, or splanchnic congestion as claimed by earlier workers. As to the bearing of the results presented in the present paper on the numerous theories of sleep, which have been elaborated by various authors, it need only be said that they speak against the view that the cardio-vascular or metabolic functions are involved in the causation of the somnolent state. Such changes as occur during sleep are merely incidental to this state. For the explanation of the phenomenon of sleep we must seek other causes, such as those described by Pavlov (1923), which are not based on the assumption of changes in the general metabolic and cardio-vascular functions.

SUMMARY

The pulse rate, blood pressure, oxygen consumption, and cardiac output of three normal, young individuals were determined in the sleeping and waking states throughout the course of the night. If one compares the values of the above-named functions during sleep with those obtained a few minutes later, after awakening the subject, one finds that only the pulse rate rises as a result of awakening the subject. This is attributed to the lability of the pulse and its stimulation by the optic and acoustic stimuli which are active when the subject is awake. The sleeping state *per se* is not associated with a decrease in the blood pressure, oxygen consumption, or cardiac output, as compared to the waking state under completely relaxed conditions at the same hour of the night. These functions assume *sub-basal* values (*i. e.*, lower than the minimum values obtained under optimum conditions of rest during the day) after 5 or 6 hours of sleep. The observed changes in the cardio-vascular and metabolic func-

[6] Gessler (1925) considers the diurnal temperature variations (which Benedict and Snell (1903) showed to be unaffected by reversing the daily and nightly activities), as a manifestation of a greater excitability of the regulatory apparatus during the day than during the night. Such a variability on the part of the general bodily activities might also be considered as the causal factor of the changes observed in the present work.

tions during sleep are either incidental to the prolonged bodily rest associated with this state or are a manifestation of cyclic changes in the organism of the nature of a diurnal rhythm.

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EFFECT OF EPINEPHRINE ON SUGAR UTILIZATION IN ANIMALS UNDER AMYTAL ANESTHESIA

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In a recent communication Colwell and Bright (1) reported that carbohydrate oxidation was suppressed completely by prolonged intravenous administration of epinephrine in spite of a constant intravenous supply of glucose. These experiments which lasted for 8 to 14 hours were carried out on cats under amytal anesthesia. Since glucose oxidation persisted under identical conditions without epinephrine, the authors concluded that the use of amytal anesthesia was probably not responsible for their finding. In order to explain the marked difference of their results to most of the experimental work dealing with this problem, Colwell and Bright pointed to the prolonged administration of epinephrine in their experiments. Curiously enough, the only previous work in which epinephrine had been found to suppress carbohydrate oxidation to a marked degree was also performed on anesthetized animals, though the period of epinephrine action was only 1 to 2 hours. This refers to the work of Wilenko (2) on urethanized rabbits. Furthermore, it has been found in this laboratory that when glucose was supplied to rats under amytal anesthesia at a rate of 100 mgm. per hour and epinephrine was injected, about 75 per cent of the amount of glucose injected remained unutilized though the experiment lasted only 1 hour (3). Under the same conditions but without epinephrine injections amytalized rats utilized 200 mgm. of glucose per hour. No determination was made of the amount of carbohydrate oxidized but it seems clear that the marked decrease in glucose utilization which was observed after the epinephrine injection was due in part to a suppression of glucose oxidation. In contrast to this it has been found that during 4 hours of epinephrine action carbohydrate oxidation of unanesthetized and sugar fed rats was only slightly diminished. It seemed therefore possible that only in anesthetized animals can epinephrine suppress sugar utilization completely or nearly completely. The following experiments were undertaken in order to test this possibility.

EXPERIMENTAL. Male rats of a uniform stock, weighing 131 to 175 grams and starved for 24 hours previous to the experiment, were used. They were injected intraperitoneally with 5 mgm. of amytal, a leg vein

was dissected and a needle tied into it. The needle was connected by rubber tubing with a burette, graduated in 0.02 cc., containing the infusion fluid. Even flow was obtained by means of the simple device of Burn and Dale (4) which has been used successfully on many previous occasions. The infusion fluid contained 10.0 to 16.8 per cent glucose plus amytal; of the latter substance 3 mgm. were given per 100 gram rat per hour. The infusion fluid was always adjusted to a pH of 7.0. Epinephrine was added to the infusion fluid in 3 experiments, the rate of infusion being 0.001 mgm. per kgm. per minute. This is the same rate as chosen by Colwell and Bright. As they point out, Cannon and Rapport (5) found that epinephrine secretion may proceed at a rate of 0.005 mgm. per kgm. per minute during reflex stimulation of the adrenals. The quantities administered per unit of time are therefore well within physiological limits. In 3 experiments 0.2 mgm. epinephrine per kgm. was injected subcutaneously at the beginning of the infusion. This was done in order to compare the two methods of application of the hormone and because in the experiments on unanesthetized rats (6) this dose of epinephrine had been given subcutaneously. Urine was collected hourly. A small slit was made in the abdominal wall and the bladder gently squeezed out between two fingers. The body temperature of the rats was frequently measured and kept between 37.0 and 38°C. After 4 hours, immediately after terminating the infusion, one gastrocnemius muscle was dissected and was analyzed for glycogen by Pflüger's method following the procedure as recently outlined (7). Blood was drawn from the vena cava inferior. The liver was quickly dissected and introduced into a weighed flask containing 60 per cent potash. Glycogen was determined by Pflüger's method in the usual manner (8). The blood was precipitated according to Somogyi's directions (9) and blood sugar determined by means of the Hagedorn-Jensen method (10). For the determination of the glucose in the infusion fluid and urine Bertrand's method was used. Detailed protocols of two experiments are given in table 1 and all experiments are summarized in table 2.

The control experiments in table 2 show that when glucose was infused at an average rate of 228 mgm. per 100 grams per hour for 4 hours, amytalized rats excreted practically no sugar. Accordingly the blood sugar at the end of the period was only moderately elevated. Rat 4 excreted some sugar, possibly because anesthesia was too deep. Previous work (11) had shown that up to 250 mgm. can be infused without leading to an appreciable glycosuria.

The average liver glycogen of rats fasted for 24 hours is 6 mgm. per 100 gram rat (12), the average muscle glycogen is 590 mgm. per cent (7). On comparing these figures with those in table 2 it will be seen that the control animals deposited both liver and muscle glycogen. Since the muscles constitute about 50 per cent of the body weight and since previous work

TABLE 1

Hourly excretion of glucose during epinephrine action

Experiments 3 and 7. Rats weighing 154.5 and 153 grams.

Amytal supply: 3 mgm. per 100 grams per hour.

Infusion fluids contained 15.04 and 13.4 per cent glucose. Average rate of infusion for 4 hours: 233.6 and 227.7 mgm. per 100 grams per hour.

HOUR	INFUSED	GLUCOSE INFUSED PER 100 GM. RAT	GLUCOSE EXCRETED PER 100 GM. RAT	REMARKS
	cc.	*mgm.*	*mgm.*	
0–1	2.48	241.4	28.8	Experiment 3; 0.2 mgm. epinephrine per kgm. subcutaneously
1–2	2.34	227.8	110.0	
2–3	2.48	241.4	138.3	
3–4	2.30	223.8	115.2	
0–1	2.4	210.2	26.5	Experiment 7; 0.001 mgm. epinephrine per kgm. per minute intravenously
1–2	2.7	236.4	140.8	
2–3	2.66	232.9	162.9	
3–4	2.64	231.2	189.1	

TABLE 2

Effect of epinephrine on sugar utilization during constant intravenous infusion of glucose into rats under amytal anesthesia

EXPERIMENT NUMBER	GLUCOSE		EXCRETED OF INFUSED	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN	UNUTILIZED OF INFUSED	EPINEPHRINE
	Infused per 100 gm. rat per 4 hours	Excreted per 100 gm. rat per 4 hours						
	mgm.	*mgm.*	*per cent*	*mgm. per cent*	*mgm. per 100 gm. rat*	*per cent*	*per cent*	
1	787.6	300.4	38.4	350	168.4	0.316	54.6	0.2 mgm. per kgm. subcutaneously
2	917.9	255.0	27.8	692	176.4	0.370	60.5	
3	934.4	392.3	42.0	546	140.0	0.346	66.4	
5	910.4	358.6	39.4	665	98.1		71.0	0.001 mgm. per kgm. per minute intravenously
7	910.7	519.3	57.0	482	130.7	0.304	78.5	
9	910.5	337.3	37.1	700	143.8	0.376	70.5	
Average..	895.2	360.5	40.3	572	142.9	0.342	67.0	
4	913.3	44.1	4.8	285	98.9	0.779	15.5	None
6	918.8	10.0	0.9	212	192.3	0.875	7.7	
8	908.7	4.0	0.5	208	111.4	0.882	6.9	
10	906.2	3.2	0.3	158	125.0	0.906	4.1	
Average..	911.8	15.3	1.6	216	131.0	0.860	8.6	

has shown that that portion of disappearing glucose which is not converted into glycogen is undergoing oxidation, it may roughly be calculated that the control animals in table 2 could have oxidized a maximum of 63 per cent of the glucose supplied to them in the course of 4 hours.

Large amounts of glucose were excreted when epinephrine was injected. The blood sugar at the end of the period was very high; apparently excretion of sugar by the kidneys could not keep pace with the amount left unutilized. Possibly if more fluid had been injected more sugar would have been excreted and the blood sugar would have been lower. In some periods the urine contained as much as 7 to 8 per cent of glucose. As in Colwell and Bright's experiment on cats, sugar excretion in the first hour was comparatively low. It takes some time for the sugar to accumulate in the body fluids. In the 3rd hour excretion was higher than in the 2nd hour and in the 4th hour it was on an average somewhat lower than in the 3rd hour.

Hitherto unpublished experiments on the sugar content of 6 entire rats showed that at an average true blood sugar level of 302 mgm. per 100 cc., 154 mgm. of sugar could be recovered from the tissues per 100 gram rat. This indicates that the blood sugar is in equilibrium with about 50 per cent of the body weight. Since the average blood sugar value of fasting rats is 90 mgm. it can be calculated that at a blood sugar level of 572 mgm. per cent, 241 mgm. of the infused glucose were retained in the tissues. Adding the amount excreted in the urine, one finds that 67 per cent of the amount of glucose infused remained unutilized in the rats receiving epinephrine as compared to 8.6 per cent in the controls. Of the 33 per cent which according to this calculation were utilized in the former animals, 15.3 per cent are accounted for by glycogen deposition in the liver. This leaves 17.7 per cent of the total amount of glucose injected which could have undergone oxidation, while the figure arrived at for the controls was 63 per cent.

The liver glycogen of unanesthetized rats after 4 hours of glucose absorption was found to be higher when epinephrine was injected than in uninjected controls (6). During amytal anesthesia this effect of epinephrine is but slight (table 2) or may be absent (12). Under amytal anesthesia no muscle glycogen was deposited when epinephrine was injected; in fact, muscle glycogen disappeared to a considerable extent (table 2). It may be assumed that a large part of it was changed to lactic acid (13).[1] In unanesthetized and glucose fed rats, though less muscle glycogen was found when epinephrine was injected, still a fair amount was synthesized (6).

[1] It is possible that a portion of the disappearing muscle glycogen underwent oxidation and that part of the lactic acid formed from muscle glycogen was resynthesized to glycogen in the liver. In either case the calculated value for carbohydrate oxidation would not be changed enough to alter the significance of the results.

The results which Colwell and Bright obtained on anesthetized animals are confirmed. These authors point out that the existing belief concerning the effect of epinephrine on glucose oxidation is mostly based on observations following a single subcutaneous dose. Such an injection cannot lead to a sustained epinephrine effect and this is the explanation of the fact that a suppression of glucose oxidation has not been observed before. It can be calculated from table 2 that a single subcutaneous injection of 0.2 mgm. per kilo[2] produced an average inhibition of glucose utilization of 60.3 per cent, while the figure found for a continuous intravenous injection at a rate of 0.001 mgm. per kilo per minute was 72.6 per cent. A subcutaneous injection can therefore produce an inhibitory effect of 4 hours'

TABLE 3

Comparison of the effect of epinephrine injection on glucose utilization in unanesthetized and anesthetized rats

Per 100 gram rat per 4 hours

	GLUCOSE ABSORBED OR INJECTED	GLUCOSE EXCRETED	BLOOD SUGAR	PER CENT OF TOTAL UTILIZED	PER CENT OF TOTAL DEPOSITED AS LIVER GLYCOGEN
	mgm.	*mgm.*	*mgm. per cent*		
Unanesthetized:					
Controls	1,065		158	98	18
0.2 mgm. epinephrine per kgm. subcutaneously	911	55	198	89	23
Amytal anesthesia:					
Controls	912	15	216	91	14
0.2 mgm. epinephrine per kgm. subcutaneously	880	316	529	40	18
0.001 mgm. epinephrine per kgm. per minute intravenously	910	405	616	27	13

duration nearly as marked as that resulting from a sustained intravenous injection of epinephrine. The difference in the results obtained on anesthetized and unanesthetized animals therefore cannot be traced to a difference in the mode of administering the hormone.

In table 3 the averages of 2 previous series of experiments on rats are recorded (6). The conditions were the same as in the present series of experiments with two exceptions. Anesthesia was not used and glucose was not supplied by vein but was fed by stomach tube at the start of the

[2] Evidence has been presented on previous occasions (6) (14) that after the injection of such a dose the effect of epinephrine on carbohydrate metabolism persists for about 4 hours.

experiment in such quantities that absorption could proceed for 4 hours. Since it has been shown repeatedly that glucose is absorbed from the intestine at a constant rate, the different route of entrance of the sugar into the body in the two groups of experiments is of little consequence.[3]

The same dose of epinephrine which causes an excretion of 55 mgm. of sugar in 4 hours and a final blood sugar of 198 mgm. in unanesthetized animals, leads to the excretion of 316 mgm. and to a blood sugar of 529 mgm. in anesthetized animals. Amytal alone lowers the ability of the tissues to dispose of glucose but slightly, as is shown by an excretion of only 15 mgm. out of 912 mgm. of glucose injected. However, when epinephrine is given to amytalized animals their power to utilize glucose is greatly diminished. The effect of the two substances is not additive, but synergistic. This may explain why it is only in anesthetized animals that epinephrine has been found to suppress glucose oxidation completely. Another possibility is that amytal merely hastens the depressive action of epinephrine on carbohydrate utilization and that after very prolonged administration and suitable dosage a marked decrease in utilization might also be demonstrable in unanesthetized animals. At any rate, it is clear that the results obtained on anesthetized animals are only quantitatively but not qualitatively different from those obtained on unanesthetized animals. It is also necessary to point out that it has not yet been decided whether the decrease in carbohydrate utilization after epinephrine injections is due to a peripheral action of the drug or to a suppression of insulin secretion by the pancreas.

The return of the blood sugar to normal after an intravenous injection of epinephrine is markedly delayed in animals under amytal anesthesia as compared to unanesthetized animals, a factor which has been disregarded by Dvorkin (16) in the interpretation of his recent experiments on amytalized cats. The conclusion that his results more nearly correspond to the physiological activity of the adrenal glands is therefore not warranted.

SUMMARY

Amytal anesthesia, which in itself has but a slight effect, greatly intensifies the depressive action of epinephrine on glucose utilization.

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[3] It was at first tried to feed glucose to the anesthetized animals but preliminary experiments showed this to be inadvisable since under amytal the rate of intestinal absorption is reduced. A similar observation has been made by Olmsted and Giragossintz (15) on dogs.

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DOUBLE ADRENALECTOMY AND THE OESTROUS CYCLE IN THE WHITE RAT

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Contradictory results have been reported on the effect of double adrenalectomy on the oestrous cycle of the rat. Wyman (1928) claims that there is a partial or total inhibition correlated with the degree of insufficiency. On the other hand del Castello (1928) found that the oestrous cycle was not affected in six rats which were studied for three months.

TABLE 1

Length of cycles in days

NUMBER OF ANIMALS	BEFORE OPERATION	AVERAGE	AFTER OPERATION	AVERAGE
1	5, 7, 5, 5, 5	5.4	5, 5, 4, 5, 4, 6, 7, 4, 4, 3, 5, 5	4.8
2	5, 6, 5, 4, 4	4.8	4, 7, 5, 4, 7, 4, 4, 4, 5, 3, 3, 2, 4	4.3
3	4, 3, 4, 5, 3, 2	3.5	6, 4, 3, 6, 4, 5, 3, 4, 3, 5, 3	4.2
4	2, 2, 3, 4, 5, 3, 6, 5	3.8	8, 3, 5, 3, 3, 6, 4, 5, 3, 4	4.4
5	3, 7, 3, 3, 4	4.0	3, 3, 4, 3, 3, 4, 3, 5, 4, 4, 5, 5, 6	4.0
6	4, 4, 3, 3, 3, 5, 4	3.7	4, 4, 7, 4, 4, 3, 4, 5, 3, 3, 2, 4, 3, 5, 5	4.0
7	4, 4, 5, 3, 6, 4	4.3	9, 4, 4, 5, 3, 4, 5, 4, 4, 3, 5	4.5
8	5, 3, 5, 3, 5, 7	4.7	4, 3, 3, 4, 4, 4, 3, 5, 4, 6, 6	4.2
9	5, 4, 4, 5, 6	4.8	5, 4, 4, 5, 4, 3, 3, 4, 3, 5, 4, 6, 6	4.3
10	4, 5, 4, 5, 6	4.8	6, 8, 4, 2, 4, 7, 4, 4, 6, 3, 5, 5	4.8
11	4, 3, 5, 3, 4, 5, 2	3.7	4, 5, 4, 5, 3, 3, 4, 6, 5, 5, 3, 5,	4.3
12	6, 3, 3, 2, 4, 4	3.7	7, 5, 3, 5, 5, 3, 4, 4, 5, 3, 3, 3, 3, 5, 4	4.1
13	7, 3, 3, 3, 2, 5	3.8	5, 6, 5, 4, 9, 3, 4, 5, 7, 4	5.2
14	6, 3, 8, 4, 4, 5	5.0	5, 4, 3, 4, 5, 4, 4, 5, 3, 5, 4. 4, 5	4.2
		4.26		4.38

We have reinvestigated this problem using the vaginal smear method of Long and Evans (1922). The oestrous cycles of 14 virgin rats, 95 days old, were studied for three to four weeks in order to obtain their normal length. The rats were then double adrenalectomized and the cycles determined for 8 to 9 weeks. The results of this study are shown in table 1. Although there are individual variations, the average length of the cycles before operation was 4.26 days, while after adrenalectomy it was 4.38 days.

At the end of the experiment, a post mortem was made and adrenal rests were found in each of these rats. This caused us to repeat the experiment with 9 other females. Before operation the average length of the oestrous cycles in the second group was 4.63 days while during the first 3 weeks' period after double adrenalectomy it was 4.72 days. Six of these animals died within the three weeks of adrenal insufficiency; regenerated "rests" were found in one of the surviving rats at post mortem, three weeks after operation.

The experience of this laboratory indicates that when rats survive double adrenalectomy more than about three weeks adrenal cortex "rests" are always to be expected.

The differences before and after operation are so slight it is clear that double adrenalectomy did not appreciably affect the length of the oestrous cycles in these two groups of rats.

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STUDIES ON VENOUS PRESSURE

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In comparison with the large amount of experimental work on arterial pressure under various conditions, venous pressure has been studied but little. Our major interests in the present study were two, first the relation, under various conditions, of arterial and venous pressure, and second, the relation of venous pressure within the heart to that in the peripheral veins. According to the view long held by physiologists, systemic venous pressure represents mainly the remains of the energy transmitted to the blood by the contraction of the left ventricle. Most of this energy is lost by conversion into heat due to friction, but a small part remains as the kinetic energy of velocity and the potential energy of venous pressure. This explanation of venous pressure would suggest a more or less definite relation between arterial and venous pressures at all times, a finding indeed of a certain school of recent investigators who have described a ratio between the two pressures in man which varies little under different conditions (1). Modern work in cardiodynamics has served however to modify to a marked degree this older conception, and to ascribe the main factor determining the pressure in the veins to the extent to which the heart empties the venous system rather than to the extent to which it fills the arterial system. This conception is founded in the main upon two physiological factors, the direct relation existing within wide limits between the venous load and the ventricular output and the variability of the peripheral vascular bed as to volume. To variations in resistance in the peripheral vascular system, recognized by the older physiologists, have been added variations in total capacity.

The question as to whether or not the pressure as recorded from an outlying vein is a true measure of the pressure at the venous end of the heart has both experimental and clinical interest. From the latter standpoint particularly, question has been raised in recent years regarding the influence of thoracic conditions (2), and it has been contended that certain abnormal states, particularly emphysema, may so disturb the usual relation between the central and peripheral venous pressures as to make determination of the latter useless as a clinical procedure.

HISTORICAL. The literature concerning experimental and clinical venous pressure has been recently reviewed in some detail (3), and will not

be repeated here. The most extensive previous work from the experimental standpoint is that of Plumier (4). He noted especially that vasoconstriction and vasodilatation altered venous pressure little unless cardiac activity was affected and that venous pressure regained its normal level in most instances sooner than arterial pressure subsequent to procedures tending to disurb the balance of the circulation. Hochrein (5) has shown that temporary occlusion of the thoracic aorta leads, instead of a fall, to a slight rise in venous pressure in the femoral vein, probably to be ascribed to accompanying venomotor changes.

METHOD. Venous pressure was recorded simultaneously in dogs from the right auricle, left iliac and left external jugular veins. Cannulae were introduced into short lateral branches of the veins, and through the right external jugular a metal sound was passed into the right auricle. These connected with water manometers and reservoirs containing salt solution, by means of which the venous pressure was balanced against a water column. Great care was taken at all times that a free communication existed between vein and manometer, since this is extremely important in the direct determination of venous pressure. The manometer tubes were connected in turn at their tops with recording tambours of rather large volume, which traced a record of venous pressure changes. The numerical changes in venous pressure were read frequently during the experiments from scales attached to the manometers and calibration of the tambours against known pressures checked against these readings.[1] Mean arterial pressure from the femoral artery and respiratory movements or intrathoracic pressure were also recorded in all experiments. The animals were under morphine-ether anesthesia. Procedures designed to alter arterial pressure, venous pressure, intrathoracic pressure, peripheral resistance and cardiac activity were then carried out. The procedures are detailed in the results.

RESULTS. In general it may be said that although the same procedure carried out in the same animal at different times or on different animals tends to produce similar effects on venous pressure measured in the same vessel, there are variations, and the effect upon venous pressure is less accurately predictable than the effect upon arterial pressure. Further, the venous pressure may show temporarily opposite changes in different parts of the venous system. As an example, stimulation of the central end of the right vagus nerve (fig. 1) with both vagi cut caused a rise of arterial pressure varying between 8 and 42 mm. of mercury in nineteen of twenty-two trials in eight animals. In the nineteen instances in which arterial pressure rose, auricular pressure rose in seventeen but was unchanged in two (when

[1] This calibration is as follows: Auricular manometer, 1½ mm. of change corresponds to 10 mm. of water pressure; iliac and jugular manometers, 2½ mm. of change correspond to 10 mm. of water pressure.

arterial pressure rose 12 and 16 mm. respectively). The rise varied from 2 to 35 mm. of water and averaged 19 mm. The iliac venous pressure on the other hand rose in six (average of 12 mm. of water), fell in eleven (average of 23 mm.), and was unchanged in two. The pressure in the jugular

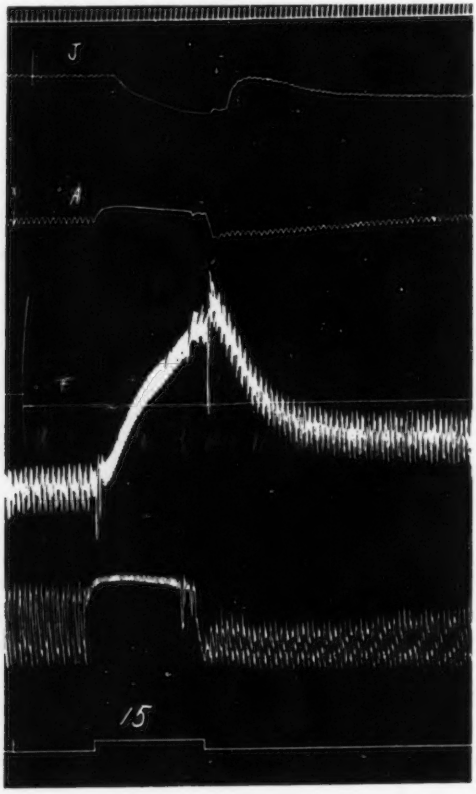

Fig. 1. Effect on venous pressures of strong central vagus stimulation in the dog. Top curve records pressure in the jugular vein, the next curve pressure in the right auricle, the third curve pressure in the iliac vein, fourth curve pressure in the femoral artery, fifth curve intrathoracic pressure. Time in one second intervals.

vein showed an approximately equal tendency to rise slightly or fall slightly (average of 9 mm. in the former and 5 mm. in the latter).

Peripheral vasoconstriction induced by intravenous adrenalin injection (fig. 2), was associated usually with effects upon auricular and iliac venous pressure exactly opposite to those observed on stimulation of the central

end of the vagus. In fourteen experiments the average peak rise of arterial pressure was 74 mm. of mercury. Auricular venous pressure instead of rising, as occurred in most instances associated with vasoconstriction due to stimulation of the central end of the vagus, fell in eleven instances (average fall of 16 mm. of water). Iliac venous pressure rose in twelve trials (average of 17 mm.), again in contrast to the former case. The effect on jugular pressure was again slight and inconsistent.

In addition to vasoconstriction, common to both of the above procedures, central vagus stimulation stops respiratory activity, and adrenalin after

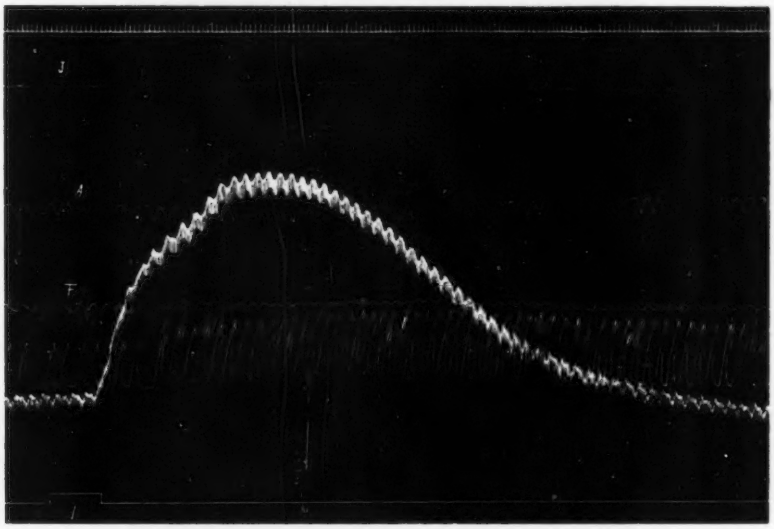

Fig. 2. Showing the slight venous pressure changes associated with extensive rise of arterial pressure following injection of adrenalin. Arrangement of curves as in figure 1.

vagus section may cause cardiac acceleration or augmentation. That the first two factors played no part in the venous pressure changes noted is evident, first from the fact that mild vagus stimulation sufficient to stop respiration for a period of 15 to 20 seconds, but insufficient to cause an increase in arterial pressure, had no effect on venous pressure, and second, that the change in heart rate under adrenalin in our experience was small, amounting to not more than a few beats per minute. The difference is probably to be ascribed to increase of stroke volume under the influence of adrenalin.

Vasodilatation induced by the inhalation of amyl nitrite in three animals,

caused a fall of arterial pressure of 16, 24 and 18 mm. of mercury respectively. In two instances auricular venous pressure was unchanged; in one it rose 5 mm. of water. Iliac and jugular venous pressures showed practically no change.

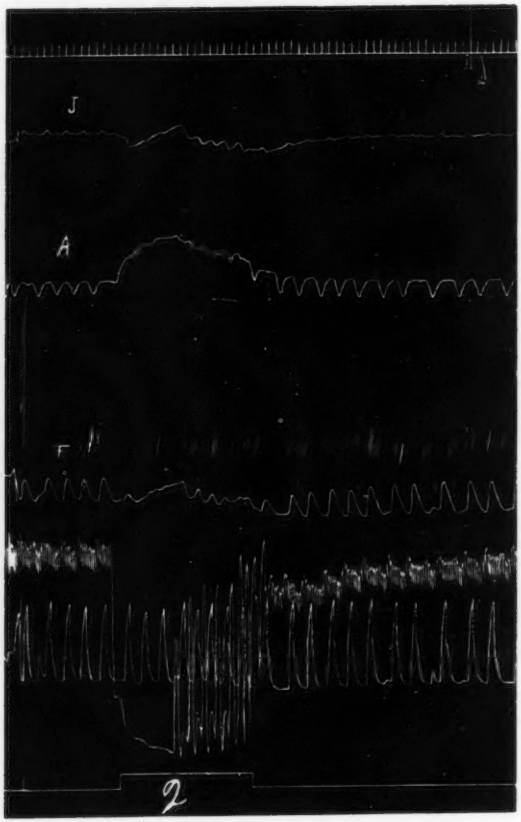

Fig. 3. Showing the slight changes in venous pressures associated with marked fall of arterial pressure resulting from peripheral vagus stimulation. Arrangement of curves as in figure 1.

It seems obvious from these results that venous pressure bears no direct relation to arterial pressure, and that it may show opposite changes in different parts of the venous system during a rise of arterial pressure. It is further evident that these changes are small and that rapid readjustment occurs with a return to normal pressure. The relative independence of

arterial and venous pressures is well illustrated in figures 2 and 3, which show examples of massive changes in arterial pressure with slight and temporary alterations in venous pressure. As a rule auricular venous pressure is somewhat more affected than iliac or jugular pressure, but the effects here are usually quite transitory.

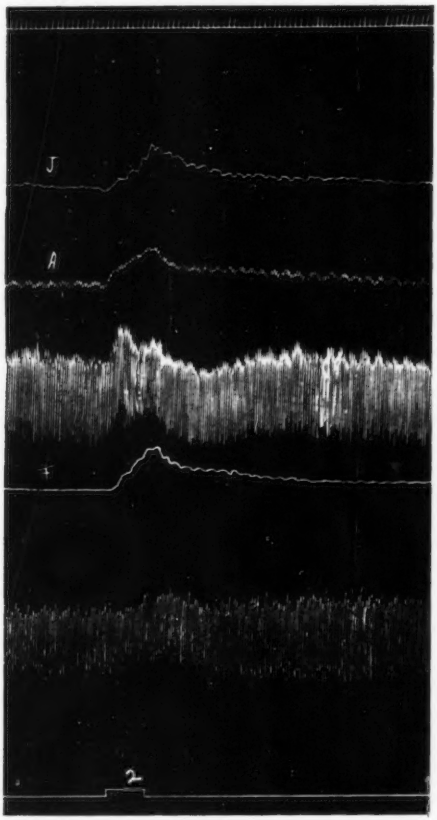

Fig. 4. The effect on venous and arterial pressures of sudden massive infusion into the right auricle. Arrangement of curves as in figure 1.

The most marked temporary alterations in venous pressures that we have obtained occurred under two procedures, sudden alteration of intrathoracic pressure by forcibly expanding or collapsing the lungs by over-filling with air or by suction, and sudden overloading of the venous system by massive infusion of saline solution (fig. 4). In two animals in which the inflation

of the lungs caused a rapid fall of arterial pressure, the auricular pressure rose 70 and 120 mm. of water respectively, the iliac pressure 12 and 50 mm., and the jugular pressure 12 and 15 mm. These changes were replaced by changes of less extent in the opposite direction as the pressure in the lungs was released. Partial collapse of the lungs by suction with the associated

TABLE 1

Effect of various procedures on the pressures in the right auricle, jugular vein and iliac vein

PROCEDURE	MEAN ARTERIAL PRESSURE	AURICULAR VENOUS PRESSURE	PRESSURE IN JUGULAR VEIN	PRESSURE IN ILIAC VEIN
Stimulation of saphenous nerve	Moderate rise	Slight fall	Unchanged	Slight rise
Asphyxia from clamping trachea	Rises, then falls	Falls slightly and then rises as heart fails	Unchanged and then rises as heart fails	Unchanged and then rises as heart fails
Artificial respiration with bellows	Falls slightly	Rises moderately	Rises slightly	Rises slightly
Increased intrathoracic pressure	Sharp rise, then marked fall	Rises moderately	Rises slightly	Rises moderately
Diminished intrathoracic pressure	Marked fall	Moderate fall	Slight fall or unchanged	Slight fall
Stimulation of peripheral vagus	Very marked fall	Moderate rise	Slight fall	Slight rise or fall or unchanged
Adrenalin (vagi cut or intact)	Very marked rise	Moderate to slight fall	Slight fall or or rise	Slight rise
Amyl nitrite	Marked fall	No change	No change	No change or slight fall
Inhibition of respiration by stimulation central vagus	No change	No change	No change	No change
Strong central vagus stimulation	Moderate to marked rise	Moderate to slight rise	Slight fall or slight rise	Slight fall
Injection of warm saline into right auricle	Slight rise	Moderate rise temporary	Moderate rise temporary	Moderate rise temporary

fall of intrathoracic pressure in three animals caused a moderate transitory rise in arterial pressure, a fall of auricular pressure of 250, 200 and 100 mm. of water respectively, and a fall of iliac pressure of 30, 10 and 40 mm. Jugular pressure was unchanged. The infusion of 500 to 600 cc. of saline solution through a large cannula in the jugular vein directed toward the

heart was carried out in several experiments and in each instance caused a sudden sharp rise in all three venous pressures, which were quickly readjusted, however, to the normal level. An example is given in figure 4 in which the auricular pressure rose 60, the iliac pressure 40, and the jugular pressure 40 mm. of water.

Table 1 gives the usual results obtained in the various procedures carried out. It should be noted again that there are many exceptions, and the only consistent characteristic appears to be the relatively slight and transitory changes in venous pressure that are observed.

CONCLUSIONS

Venous pressure in the dog under anesthesia tends to show relatively small and transitory changes under conditions which alter markedly the physical conditions existing in the cardiovascular system. There appear to be compensatory factors present tending to prevent excessive changes and to cause rapid restoration to a normal level.

There appears to be little if any direct relation between arterial pressure and the pressures in different parts of the venous system. Inverse changes occur as frequently as direct, and both may be present simultaneously if different parts of the venous system are considered.

In general the pressure is more stable and less subject to alteration in the peripheral veins (iliac and jugular), than in the right auricle. There appears to be some factor or factors tending to reduce the extent of change arising at the heart as measured further out in the vascular system. Not infrequently inverse changes may occur in the periphery as compared with the auricular pressure change, but in all cases the result is transitory and rapid readjustment occurs.

These results tend to emphasize the fact that the vascular system, particularly the venous part, cannot be reduplicated physically by a simple hydraulic model. Many factors, some unknown and some only partially recognized, modify markedly the comparison to a more or less static system of channels. Two of these factors, the varying response of the heart to varying venous loads and the variable capacity of the vascular bed, would seem to be of essential importance.

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STUDIES ON THE MECHANISM OF WATER EXCHANGE IN THE ANIMAL ORGANISM

I. The Nature and Effects of Superficial Burns

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A previous communication (1) outlined a new conception of the mechanism involved in the production of a portion of the systemic effects induced through the agency of extensive superficial burns in man. The essential feature reported is that under these circumstances the blood becomes highly concentrated. The concentration of the blood is ascribed to a loss of fluid on to the body surface through increased permeability of the injured capillaries of the skin. Upon this conception a method of treatment was based which yielded markedly successful results. Naturally this experience with burned cases in man gave rise to numerous questions which for their solution demanded the employment of experimental animals.

Preliminary work soon demonstrated that a burn on the skin of the ordinary experimental animal produced results differing somewhat from those commonly encountered in man. This difference in certain directions is of great advantage in studying the changes induced without altering essentially the applicability of the results obtained to similar conditions in man. The noteworthy difference mentioned is that a burn in the usual experimental animal fails to produce a blister which is characteristic of a burn in man. It would appear that in the experimental animal it is almost impossible by an inflammatory reaction to separate the epidermis from the dermis. It is quite possible that this is associated with the absence of functional sweat glands in the skin of the experimental animal. A similar experience was noted in the study of the production of a blister by the so-called "mustard gas" during the World War. In order to investigate the nature of the mustard gas blister it became necessary to study the action of this substance on the skin of man. In this connection the follow-

[1] A portion of these data are taken from the dissertation presented to Yale University by Merl E. Fisk in candidacy for the degree of Doctor of Philosophy, June 1929.

ing statement by Hanzlik (2) may be quoted: "So far as known the skin of animals does not blister. Mild effects of irritants on the skin of animals are indicated by simple hyperemia, rashes, moderate swelling and edema, also moderate petechial hemorrhages. Instead of vesication, the more severe effects are characterized by rather extensive edema and swelling, and the severest effects by gangrene and ulceration.

"On the other hand, the acute and more severe effects on human skin are characterized by hyperemia and vesication, sometimes pustulation, and the severest effects also by ulceration. The scars in the skins of different species do not differ greatly, and depend upon the degree of ulceration and extent of destruction of tissue." "The part played by the sudoriparous and sebaceous glands on the entry (absorption) of the compounds into the skin was also not studied. In this connection it is interesting to note that horse's skin, which contains sweat glands, was found to be more sensitive to a number of compounds than the skin of men, dogs and monkeys."

In view therefore of our confirmatory experience with respect to blister formation we have chosen the rabbit as the experimental animal for our study of burns. At first, acting upon the suggestion of Robertson and Boyd (3), half-grown animals were selected because of the apparent greater susceptibility of the skin. Experience proved, however, that full-grown rabbits are just as desirable as those younger.

EXPERIMENTAL. *Technic of producing a burn.* For the production of a burn a small-sized electric flat iron was employed. With this a maximum heat is attained which remains fairly constant so long as the current is passing. In preparation of the application of the hot iron the skin was either shaved or clipped with scissors free from hair. The animal was then completely anesthetized with ether and the iron applied. The time of application of the iron varied from a few seconds to a minute or more according to the type of burn desired. In the production of a burn the iron was never allowed to come to rest but was slowly passed over the area selected. The site usually selected was the ventral surface extending from the xiphoid process to within a few centimeters of the groin and latterly almost to the mid-axillary line. At times, however, other sites were selected, for example, the skin covering the back, the line of injury extending down to the mid-axillary line. In general, these burns were of the first and second degree involving all the layers of the integument and covering an area approximately one-seventh to one-fifth of the total surface of the body. In certain experiments third degree burns were produced.

When the burning was completed the animal was allowed to recover from the anesthetic. Within a period of less than one-half hour the animal had regained its normal behavior and appearance and usally presented no symptoms indicative of shock. When burns were produced on the ventral surface shock was never in evidence. When, however, burns were

induced on the dorsal surface, particularly when the iron passed over the spinal column, shock-like or various neurological symptoms were likely to occur followed in some instances by death. If the skin is drawn to one side and burned, no nervous disorders are in evidence. It would appear therefore that the deleterious symptoms observed by burning the back are referable to injury to the spinal cord. After the burn has been produced no evidence of pain is present. Even on stimulation of the burned area no pain reflex can be elicited. To all appearances general normal conditions are resumed as soon as the effects of the anesthetic have worn off.

It is probable that in man the shock characteristic of an extensive superficial burn is due to pain and to various other reflexes and psychological factors as fright, etc. These are absent in the anesthetized animal, consequently no shock is evident. Early deaths, that is within 24 to 48 hours, rarely occurred. Late deaths were ascribed to secondary infection or to injury to abdominal viscera, usually the latter. Clinical experience has demonstrated that the extent of the burn plays a distinct rôle in the outcome. If more than one-third of the body surface is involved death is likely to occur. The paucity of early fatalities in our experiments may be ascribed in great measure to the fact that less than one-third of the body surface was injured.

The character of the burn. When the skin of the rabbit is burned to a degree beyond a condition of simple erythema, there develops within a period of two hours a marked swelling and edema of the injured area. The edema fluid finds its way into the fascia, carrying the blood vessels, adherent to the inner surface of the skin and is there held as a subcutaneous tumor mass. This subcutaneous edema occurs over the entire injured area but tends to be most abundant on the periphery of the wound. If the injured area is excised and suspended the subcutaneous edema hangs beneath in a series of pointed finger-like projections, similar to the stalactites in some caves, from which fluid slowly oozes. The fluid when the mass is cut from the body, soon assumes a jelly-like consistency due to coagulation. Usually the fluid is clear and translucent. The maximum edema occurs during a period of 24 to 48 hours, after which it is reduced in amount. The mass becomes opaque and shot through with blood indicating that organization is taking place. As organization proceeds the growth of fibrous tissue gives the exudate increased toughness while the skin itself grows paper thin preparatory to sloughing; it is then possible to peel the organized tissue from the skin with great ease.

At any stage in the development of the subcutaneous edema mass it is possible to separate it from the skin by cutting with a sharp razor or scissors. Of course included in this mass is the fascia which, however, in the rabbit is very scanty.

The skin itself soon after the burn presents a cooked opaque appearance which gradually turns brown or black and eventually becomes thin and parchment-like with a tendency to stiffen with time. After a period of 3 to 4 days the burned area presents an appearance of complete necrosis and a slough forms, which gradually becomes elevated, contracts, and is cast off within a period of two to three weeks. The injured area finally contracts to such a degree that no trace of the wound may be seen beyond a transverse line of what is apparently scar tissue.

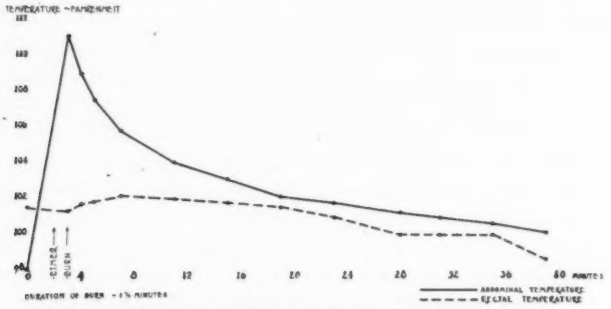
Usually the muscle below the wound shows erythema and edema, the whole presenting a swollen, opaque appearance. If the burn has occurred on the ventral surface of the body the vessels of the intestines may have an initial congested aspect and there may be edema of the intestinal walls. In some instances areas as large as a dime may be found on the stomach and intestines indicating that the heat has penetrated to these tissues. These areas may become necrotic and lead to perforation. Or in other instances the injured spot adheres to the abdominal wall and eventually a fecal fistula is formed ultimately causing death.

The influence of a burn on temperature changes in the body. In the literature are many statements (4) calling attention to the presence of hemorrhages, ulcerations, necroses, etc., in the abdominal organs after a burn. The character of the pathological changes is of a nature such as to lead one to assume that the fundamental defect common to all lies in a disturbed circulation, generally, however, restricted in area. So far as the present authors are aware little attention has been paid to the possibility that direct heat penetration to the abdominal organs may play a rôle in this connection. It is not at all unreasonable to assume that an elevated intra-abdominal temperature may seriously interfere with the local circulation in the areas in the path of the direct heat penetration. We know of no previous attempt to measure such possible temperature changes and consider such data of great significance in the solution of the problem before us.

Accordingly rabbits were anesthetized with ether; a thermometer was inserted through a small slit deep into the abdominal cavity and another was placed high into the rectum. The iron was applied to the usual abdominal area for varying periods of time and the temperature noted on each thermometer at intervals of one minute for one-half hour or more.

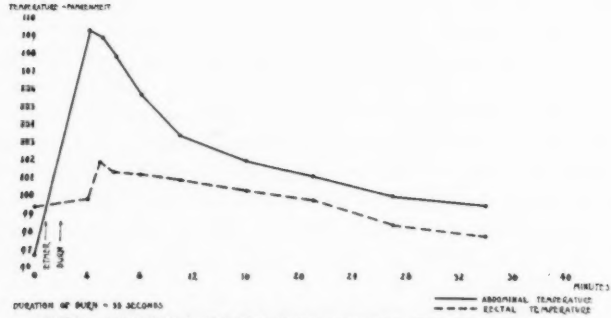
That markedly increased temperature may be induced within the abdominal cavity by relatively short periods of burning may be observed from charts 1 and 2 which are typical of other similar experiments. The height of the augmented temperature is directly related to the period of time during which the iron was in contact with the skin. The response in abdominal temperature to the application of the iron is rapid, and then

there is a quick fall, the temperature gradually reaching an approximately normal level within 20 to 30 minutes. The temperature within certain areas of the abdominal cavity may exhibit even greater elevations than those recorded in the chart. For example, if the thermometer is pressed upward against the inner surface of the abdominal wall instead of being deep down in the cavity the temperature reached may be much higher



INTRA-ABDOMINAL TEMPERATURE CHANGES DUE TO BURNS

Chart 1



INTRA-ABDOMINAL TEMPERATURE CHANGES DUE TO BURNS

Chart 2

than that given. It is quite obvious that such high temperature, even though momentarily maintained, must definitely injure such delicate structures as the intestines.

Most, if not all, the macroscopic appearances that have been described as characteristic of burns have been noted in our experimental animals. This, however, is true only when the heat has penetrated the abdominal cavity. If the burn has been remote from this region nothing abnormal

has ever been noted except possibly some congestion. The congestion observed under these circumstances may be explained on the basis of altered circulation due to a markedly concentrated blood (see later). Similar congestion has been observed in conditions characterized by concentrated blood, particularly in irritant gas poisoning (5).

In view of these facts the conclusion seems inevitable that heat penetration to the abdominal cavity plays a significant rôle in the production of those pathological appearances usually attributed to a toxemia from the burned area.

Corresponding to the alterations in abdominal temperature are changes in the rectal temperature (see charts 1 and 2), which, however, never approach the level attained by the former. The rise in rectal temperature is rapidly followed by a fall which invariably descends to a level somewhat below the initial value.

TABLE 1

Variations in rectal temperature of normal rabbits

1. 100.4	11. 100.9	21. 99.5	31. 100.0
2. 99.3	12. 101.2	22. 100.0	32. 101.2
3. 101.3	13. 101.1	23. 103.1	33. 102.2
4. 101.3	14. 100.0	24. 103.6	34. 102.2
5. 101.5	15. 101.5	25. 103.1	35. 102.9
6. 102.2	16. 101.7	26. 103.6	36. 101.3
7. 101.5	17. 101.2	27. 102.2	37. 100.8
8. 102.2	18. 103.0	28. 103.8	38. 98.9
9. 101.5	19. 101.8	29. 101.5	39. 100.0
10. 100.0	20. 103.5	30. 99.5	40. 101.7

In their paper on burns Robertson and Boyd (3) present evidence of a rapid rise in temperature 24 to 36 hours after a burn. The temperature may rise from a level of 100–102°F. to one of 104–106°F. and in the two illustrative examples given death followed on the fifth day. Robertson and Boyd apparently regard this rise in temperature as one of the toxic symptoms characteristic of burns. Our own experience with a very large number of animals has been quite different. Robertson and Boyd state that the normal temperature of the rabbit varies between 100 and 102°F. We have selected at random from our protocols the temperature figures of forty animals newly arrived from the animal farm and have tabulated the results. Table 1.

An inspection of this table will show that the temperature range varies from 98.9 to 103.8°F. It should be pointed out that these rabbits were young, about one-half grown, as were Robertson and Boyd's, in which it is to be expected that the temperature regulation is less stable than in adult animals.

In the following protocol are data concerning rectal temperature changes induced by a burn which are typically illustrative of other similar experiments.

TABLE 2

Rectal temperature changes in rabbits after a burn

DATE	TIME	TEMPERATURE RABBIT A	DATE	TIME	TEMPERATURE RABBIT B
	p.m.	°F		*p.m.*	°F.
Jan. 6	12:02	101.1	Jan. 6	12:15	101.7
	12:04	Burned		12:20	Burned
	12:10	100.4		12:25	101.3
	12:28	99.9		12:29	100.4
	12:45	100.6		12:45	101.1
	2:00	102.0		2:15	102.2
	3:00	102.7		3:15	103.1
	4:00	103.1		4:15	103.5
	a.m.			*a.m.*	
Jan. 7	9:00	103.5	Jan. 7	9:00	103.5
9		100.9	9		103.5
10		100.4	10		102.2
11		99.9	11		101.8
13		100.4	13		101.8
15		101.5	15		102.7
16		100.6	16		100.2
18		102.2	18		102.7
27		101.0	27		103.1
Feb. 17		103.5	Feb. 17		103.3
Discarded Animal in excellent condition			Discarded Animal in excellent condition		

TABLE 3

Temperature changes in control animals

DATE	TIME	TEMPERATURE RABBIT C	TEMPERATURE RABBIT D
		°F	°F
Feb. 15	12:15 p.m.	101.8	102.2
	12:16 p.m.	Ether anesthesia	Ether anesthesia
	12:45 p.m.	100.9	101.3
Feb. 16	11:00 a.m.	102.4	101.8
Feb. 17	p.m.	102.4	104.2
Feb. 18	a.m.	101.3	102.2
Feb. 20	a.m.	100.8	101.2

From table 2 it is quite apparent that immediately following a burn the rectal temperature tends to decline for a short period and then ascends to

the initial level or above. Records of temperature changes over a period of more than a month following burns demonstrate quite clearly marked fluctuations which however are always within the possible limits exhibited by this type of animal. The early initial drop in temperature may probably be ascribed to the effect of the anesthetic rather than to any influence of the burn since control animals (table 3) under anesthesia and comparable conditions except that the burning was omitted show the same tendency.

A wide experience with burns has convinced us that whenever marked and maintained temperature elevations are encountered 24 to 36 hours following a burn such high temperature may be regarded as evidence of a secondary infection of the wounded surface. The experimental data support the clinical observations made on man which led to the statement recorded in a previous communication that "the later much augmented

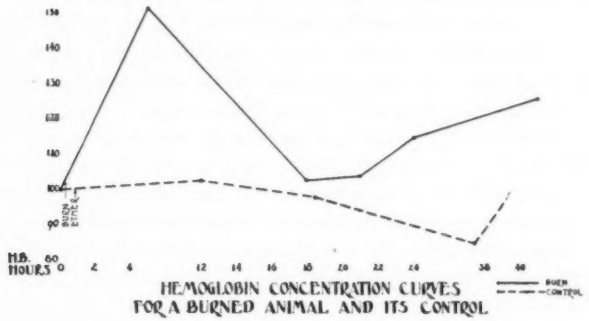


Chart 3

temperature is undoubtedly to be interpreted as a sequence of infection of the wounded areas" (1). It may be further stated that these late elevations of temperature cannot be accepted as evidence of a specific burn toxin.

Blood concentration in burns. It is to a highly concentrated blood in man (1) after extensive superficial burns that the chief detrimental systemic effects have been ascribed. In the rabbit a similar blood condition exists. In chart 3 may be seen an example of blood concentration which is typical, together with one for a control animal. The concentration of the blood increases rapidly in the first 3 or 4 hours after the burn, values 25 per cent to above normal being the rule, and then falls to normal or below 50 rising again slowly as the length of time the animal is without water increases. The light ether anesthesia employed does not of itself cause any appreciable rise in the hemoglobin concentration nor does a lack of water

alone for 72 hours. In these experiments the burns were light and over a relatively small area, insufficient to cause death. Although a marked concentration of the blood occurs, it is usually not maintained for many hours, the animal being capable of compensation, apparently by drawing upon the water reserves of the body. No attempt has been made to produce a burn of such gravity as to preclude the possibility of compensation. With burns in man there are many instances (1) where this may be observed and without doubt similar conditions may be induced in the rabbit by burning an area sufficiently large.

The measure of blood concentration has been the determination of the hemoglobin values, employing the Newcomer method. Its use as a measure of blood concentration has been justified by our own experience and that of others who have found that in general the hemoglobin changes parallel those of the total solids and the total plasma volume (dye method).

Although usually marked increases in blood concentration are to be observed, as measured by hemoglobin estimations, there are rare examples where an apparent blood dilution occurs even though there is an abundant subcutaneous edema. Or again, at first there may be increased blood concentration which is succeeded by rapid dilution below the initial level. The explanation for such facts is not at first glance entirely obvious.

The most plausible viewpoint is that these instances may be regarded as excellent examples of the ability of the organism to mobilize water. Probably water is one of the most easily mobilizable constituents of the body. In individual animals there is great variation of water storage at a given moment which is determined by a number of obvious factors. When water is abundant the first response to an inflammatory reaction may be so great as to result temporarily in blood dilution, or, if the available water supply is not great enough to accomplish this immediately or the stimulus is not sufficiently strong, blood concentration may occur but is followed rapidly by dilution, even below the normal level. Such conditions may be looked upon as examples of factors of safety resident in the organism. At times somewhat similar conditions obtain in irritant gas poisoning. This is especially true with phosgene after the inhalation of which a massive pulmonary edema results and accompanying which is generally an initial stage of blood dilution. This period is transitory and is rapidly succeeded by the second stage, that of blood concentration because of the overwhelming transport of water to the lungs. It is of interest to note also that with chlorine gas poisoning the first stage, that of dilution, is usually lacking. It is probable that in both chlorine gas poisoning and in burns the inflammatory reaction is so sudden and violent that the dilution stage is transitory, if present at all, and accordingly only blood concentration may be observed. In support of such a view is the fact that in phosgene poisoning there is always a latent period before an intense inflammatory reaction is obvious.

This ability of the organism to overcome the tendency to increased blood concentration is of great prognostic value in the treatment of the systemic effects of burns as previous experience with man has demonstrated. So long as blood concentration is not maintained above a definite high level the condition of the patient cannot be considered dangerous. It is because of their importance in prognosis that attention has been called to these unusual instances of decreases in blood concentration with burns. In the great majority of cases, however, increases in blood concentration are seen and the higher the concentration and the longer it is maintained the graver becomes the prognosis. The ultimate result is a deficient circulation with subsequent tissue asphyxiation and death unless measures are taken to reduce the blood concentration to a degree compatible with the maintenance of an efficient circulation. These views with results obtained have been outlined elsewhere (1) and need not be detailed here.

Circulatory changes in the edema mass. The formation of the subcutaneous edema affords an excellent opportunity to study the circulatory changes in the fascia carrying the edema fluid. If this fascia is carefully dissected from the skin there is obtained a very thin membrane, which when spread upon a frame of cardboard or wood, and properly illuminated, reveals the details of the blood vessels distributed to the skin. Such a preparation may be studied under the microscope or may be photographed. In figures 1, 2 and 3, are shown photographs of typical membranes of this character obtained from burned animals. Figure 1 represents the character of the circulation in the burned area after a period of one hour. It will be noted that the tissue is rich in dilated capillary vessels, which so far as one may judge present a normal appearance. There appears to be no evidence of thrombosis or of ruptured vessel walls. Compared with normal fascia that from the burned area presents a markedly inflamed appearance and apparently alters little except perhaps to become more hemorrhagic up to a period of 24 to 36 hours. Figure 1 therefore is quite typical of the usual picture obtained with a burn. In certain other instances there may be obtained circulatory changes that are markedly different from the usual appearance. Whether these appearances indicate a stage in the alterations that always occur in the circulation of a burned area and are transitory, or whether they are brought to light only under certain conditions remains a problem. In our experience they are exceptional but are not necessarily confined to the vessels in the fascia under the skin for identical appearances have been noted on the outer surface of the stomach when intense heat has penetrated to this organ. Figures 2 and 3 are typical examples of these unusual appearances. They represent a magnification of 3 times.

Figure 2 illustrates the appearances observed four hours after a burn. There are at least two structures which attract attention. In general, the

capillaries are widely dilated and seem to branch like the limbs of a tree from the main vessel. Inspection under the microscope reveals that these terminal branches are densely packed with aggregations of red blood cells. At *A* the appearance is somewhat different and may be interpreted as an extreme dilatation of the capillary with extrusion of pocket-like projections filled with corpuscles. The capillary itself is more or less empty. It would appear in these instances that some of these extrusions have broken and the corpuscles have found their way out of the lumen of the vessel. In figure 3 is the appearance noted 24 hours after a burn. Here the capillaries present for the most part the picture to which attention was directed at *A* in figure 2. One may suppose that the branching, gorged appearance of the capillaries so noticeable and widespread in figure 2, is a preliminary stage in the damage of the capillary, the almost bursting appearance being due to the fact that the plasma has largely left the capillary leaving behind the densely-packed mass of red blood cells. The predominant capillary in figure 3 is that in which apparently the corpuscles have found their way out of the lumen of the vessel either through a break in one of the budding protrusions or directly through the greatly dilated wall. In any event, it would seem that the second picture is a sequence to the first in the progress of capillary damage, and probably capillary destruction. Whatever may be the correct details concerning interpretation, the pictures afford one an excellent visual impression of the type of damage suffered by the capillary and further indicate the rôle played by the capillary in the production of the edema so characteristic of a burn.

The development of edema. After a burn the development of edema is very rapid. Even at the end of an hour the accumulation of fluid beneath the skin is quite evident. There is a steady increase in the subcutaneous edema, a maximum being reached in approximately 24 hours. In some instances this level is attained at a much earlier period, but, in general, with a large series of animals there is a steady increase to the 24 hour peak, after which conditions may remain more or less stationery for another interval of 24 hours. This is succeeded by a period during which the edema becomes gradually less and finally has almost disappeared at the end of 5 to 6 days.

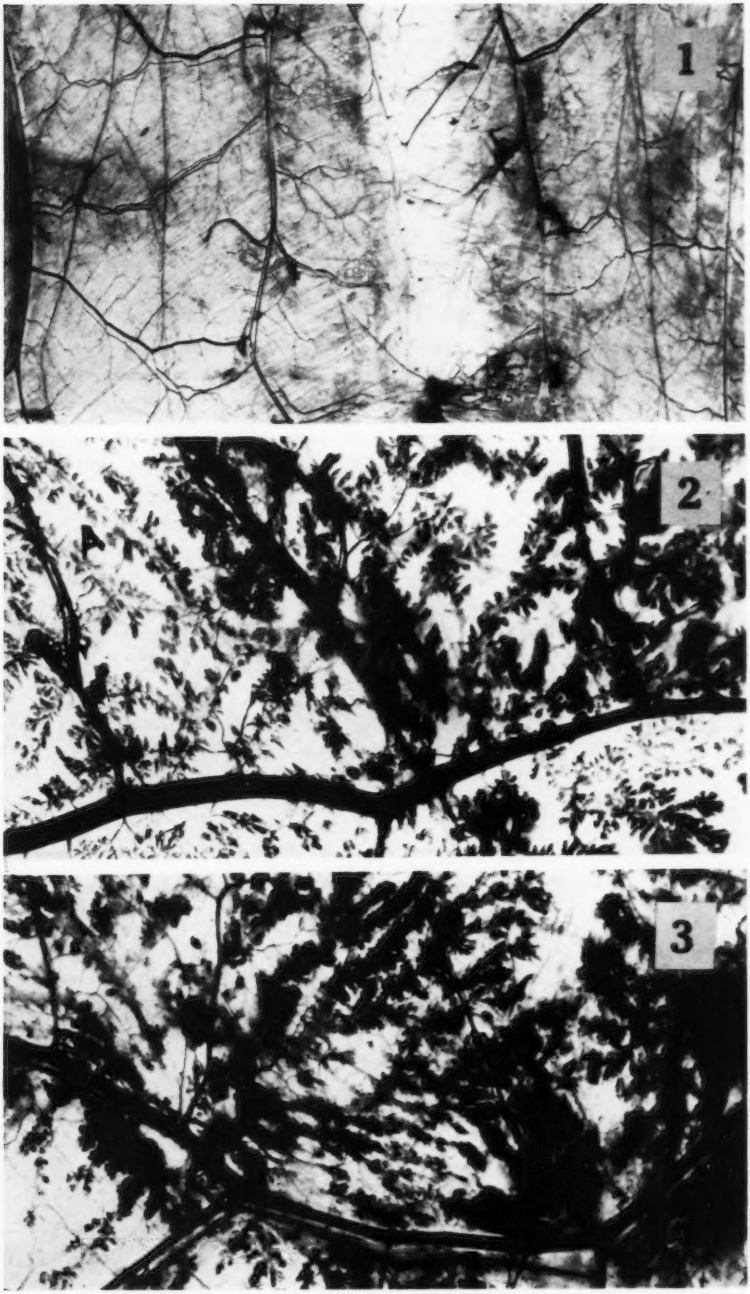
SUMMARY

When a rabbit receives a superficial burn subcutaneous edema is produced, the production of which causes a marked concentration of the blood.

Fig. 1. Photograph of circulation in fascia of skin after a burn.

Fig. 2. Photograph of circulation in fascia of skin after a burn. Magnification 3 ×.

Fig. 3. Photograph of circulation in fascia of skin after a burn. Magnification × 3.



Figs. 1–3

The development of the edema is rapid, reaching a peak at a period of approximately 24 hours. Reabsorption of the fluid material is slow being completed only at the end of the fifth or sixth day. The circulatory changes in the edema mass and the process of wound healing are described.

In the production of a burn heat may penetrate the interior of the body to a degree sufficient to increase temporarily the temperature of the body cavities by several degrees. Such an increase of temperature is regarded as sufficient to lead to local circulatory changes inducing the formation of ulcers, hemorrhages, etc., which from time to time have been ascribed to the effect of a burn toxin.

Under the experimental conditions, general body temperature is little affected by an experimental burn, and when the temperature rises later than a period of 24 hours following a burn it is probably due to infection of the wounded area.

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STUDIES IN THE MECHANISM OF WATER EXCHANGE IN THE ANIMAL ORGANISM

II. Changes in Capillary Permeability Induced by a Superficial Burn

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The development of edema under the conditions imposed by a burn is ascribed to a change in capillary permeability. The injury induced by the heat causes dilatation of the capillaries with increased ability to pass plasma through the cells, a process that may be looked upon as the first stage in the reparative process. The very rapid exit of fluid from the capillaries and the retention of the fluid in the burned areas leads to the query whether the permeability of the capillaries is changed in one direction only, namely, from within to the outside, or whether the increased permeability in the one direction is accompanied by a decreased permeability in the opposite direction, namely, from the outside to the lumen of the vessel. We have attempted to render a decision to this query by the employment of dyes and by studying the rate of absorption from the site of the subcutaneous edema and from the periphery of the injury.

1. Experiments with methylene blue. The investigation with methylene blue included two types of experiment: *a.* Rabbits were given an intravenous injection of methylene blue (10 cc. of 10 per cent solution) immediately after being burned. They were allowed to live for intervals up to a period of 120 hours. When killed, the subcutaneous edema mass was carefully separated from the skin, spread on cardboard and allowed to dry. Methylene blue in the body is in part reduced to the leuco-compound but on exposure to the air or when treated with an oxidizing agent as hydrogen peroxide quickly regains its characteristic blue color so that tissues which seemingly may be colorless eventually become blue. As a comparison for the staining of the edema mass a portion of the normal subcutaneous fascia was treated in an analogous manner. The preparation of the edema mass as outlined affords an excellent opportunity for comparative study since after drying these specimens may be kept unchanged indefinitely.

b. Rabbits received an intravenous injection of methylene blue as under *a* except that it was given at stated intervals after the burn was inflicted.

This series of animals was killed one-half hour after the injection of the dye and the preparations made under *a* were duplicated.

A study of the preparations reveals several interesting facts. Considering first the specimens obtained from animals in which methylene blue was injected immediately after burning one may conclude that the dye can be readily observed in the edema mass at an interval of one-half hour or less after the injection. In this early period the dye is distributed only on the periphery of the wounded area. Within two hours it is richly distributed over the entire edema tissue and remains more or less unchanged up to an interval of 8 hours after which it gradually diminishes in depth of color and finally is present only in traces beyond a period of 24 hours. The manner of disappearance is quite instructive from the viewpoint of throwing light upon the mechanism of absorption of the dye. Just as the dye is first seen on the periphery of the wounded area so this is the place where it is last observed. One may therefore infer that the dye is passed into the edema fluid by the capillaries of the periphery and that the capillaries of the central portion are not concerned in the dye excretion or reabsorption. The later central distribution of the dye is explicable on the ground that it gains access by diffusion from the periphery. It is quite probable that the capillaries in the centre of the burn are rendered entirely non-permeable. As the dye leaves the edema fluid, absorption occurs also from the periphery only since the central portion is first colorless, the periphery being colored even beyond the 24th hour. The edema is most abundant on the periphery and the fluid is more deeply stained here than elsewhere. The quantity of fluid in the central portion also shows signs of absorption before that on the periphery. The whole situation might be represented by a pool shallower in the centre than on the periphery. Suction exerted equally on all portions of the periphery would cause the fluid of the centre to disappear before that on the periphery. In contradistinction to this is the behavior of the normal fascia stripped from an uninjured area of the skin. The dye stains this for a period of not more than $\frac{1}{2}$ hour. The reabsorption is extremely rapid.

The outstanding features of this experiment are: 1, the fact that the periphery is the portion of the wound active in the passage of materials from the blood to the wound and vice versa; 2, the fact that the dye enters the wounded area at a much more rapid rate than it leaves. In other words, one may imagine that there are two streams, one from the blood to the wound and the other in the opposite direction, and that the first is by far the stronger and most effective in transport of substances. It is possible that the permeability of the peripheral capillaries may be more permeable for the passage of fluid in the one direction than in the other. If one considers the second type of investigation with methylene blue, namely, the injection of the dye at varying intervals after a burn, one fact stands

out clearly, namely, that in the period of survival after the dye injection not a trace of the dye was ever found in the central portion of the edema mass but on the contrary, the dye was always present in significant amounts on the periphery in all burned preparations up to a period of 120 hours beyond which the investigation was not carried.

This observation tends to support the previous conclusion that it is only on the periphery that passage of fluid in either direction occurs, at least in the early stages of the effects of a burn.

2. EXPERIMENTS WITH TRYPAN BLUE. In the investigation with methylene blue, the dye was selected for the reason that it is readily soluble in water and passes with ease through living membranes. In contrast to this is trypan blue which forms a colloidal solution with water and presumably does not pass through normal membranes readily. By comparing the behavior of these two dyes in the inflammatory reaction induced by a burn it was hoped to obtain further information concerning the relative permeability of the capillaries.

With trypan blue a procedure was followed similar to that outlined for methylene blue.

a. Rabbits received intravenous injections of trypan blue (5 cc. of 5 per cent solution) immediately after being burned and were killed at intervals, the subcutaneous edema mass being preserved and observed for the character of the stain present.

b. Trypan blue was intravenously injected into rabbits at definite intervals after a burn. They were killed at the end of ½ hour, the edema mass being treated as under *a.*

When the rabbit is given an injection of trypan blue immediately after a burn the edema mass separated at the end of an hour shows only a faint stain on the periphery; the central portion is not stained. In the two hour specimen the entire edema mass is very deeply stained. The same statement holds true for all intervals up to and including 24 hours. Later than this there is evidence that the dye is less deep especially in the central portions, the periphery still remaining deeply stained. In the later periods observed, there is an apparent progressive diminution in color. At the 120th hour the dye is markedly decreased in amount. A comparison of the different specimens shows further that the period of greatest transport of dye to the edema mass lies within the period of 2 to 24 hours. Furthermore, it would appear from this criterion that the greatest dye transport occurred in the early hours of this period rather than in the later hours. If one may assume that the depth of dye in the specimens is proportional to the permeability of capillaries then we must conclude that the greatest permeability of capillaries lies within this period of 2 to 24 hours, emphasis being upon the early hours rather than upon the later. Observations on these stained specimens further demonstrate, as with methylene blue,

that reabsorption takes place slowly. Moreover, the dye does not penetrate the capillary wall with a facility at all comparable to that in the direction from the blood to the edema mass. The capillary appears to be much more permeable for the passage of the dye from the blood to edema mass than in the opposite direction. If the dye once enters the edema mass its exit by reabsorption is very slow indeed.

In consideration of this question another aspect may be discussed, namely, the possibility that in the early hours the permeability of the capillary may be increased in both directions and that later it may tend to regain a normal ability to hold the colloidal dye within itself and also that this restoration of permeability may hold in both directions. Such a possibility would of course tend to maintain the dye in the edema fluid. A partial answer to this question is afforded by the results obtained under *b* in which rabbits received intravenous injections of trypan blue at varying intervals after a burn and were killed one-half hour later. Under these conditions a rabbit receiving trypan blue one hour after a burn yields an edema mass containing either no trypan blue or the very faintest stain on the periphery, none in the central portions. At this time it would appear that the permeability is not greatly increased—the dye has great difficulty in traversing the capillary wall. A comparable specimen obtained from an animal with a two hour burn yields a stain which has diffused throughout the entire edema mass but is not deep in color. With a four hour burn the color is still deeper and even more diffuse. At the 8th hour there is no stain at all and from this period up to 120 hours the edema mass is never stained. From such data it may well be argued that the capillaries have regained their normal permeability to a degree sufficient at least to prevent the entrance of the colloidal dye. Such a view, however, does not preclude the possibility of continued entrance of more diffusible substances for a much longer period. It does, however, give a clue to the length of time extreme capillary permeability may last.

As with the methylene blue experiments, it is quite safe to conclude that whenever transport of material takes place from the blood to the edema fluid in a burn it does it at the periphery of the wound and not in the central portions.

It should be stated that in no instance did fascia from normal portions of the skin ever show a stain after trypan blue injection.

3. THE ABSORPTION OF A SOLUBLE DYE (PHENOLSULPHONEPHTHALEIN) FROM THE EDEMA MASS. In order to study the rate of absorption of an easily diffusible substance from the wounded area, advantage has been taken of our knowledge of the behavior of phenolsulphonephthalein when injected subcutaneously into rabbits. If this dye is introduced in a dose of 6 mgm. under the skin of a normal rabbit, previously supplied with 50 cc. of water by stomach tube the elimination of the dye through the urine

is very rapid (1). The present investigation consists of three sections: *a*, injection of P. S. P.[1] into normal rabbits; *b*, injections of P. S. P. in animals with a burn, the dye being introduced under the skin remote from the injured area, usually on the back; *c*, the injection of the dye directly

TABLE 1

P. S. P. Absorption

RABBIT NUMBER	WEIGHT	FIRST HOUR	SECOND HOUR	TOTAL	24 HOURS	48 HOURS
Normal animals—50 cc. water by tube						
	K.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	2.0	64	31	95		
2	1.5	50	29	79		
3	1.8	51	32	83		
4	1.8	40	38	78		
5	2.0	50	41	91		
6	2.4	53	37	90		
Burned animals—injection of P. S. P. on back						
	TIME					
1	Immediately	40	23	63	98	
2	Immediately	33	42	75	85	
3	8 hours	40	34	74	84	
4	24 hours	69	28	97		
5	48 hours	55	32	87	97	
6	48 hours	46	40	86	96	
7	72 hours	50	38	88		
Burned animals—injection of P. S. P. in burned area						
1	Immediately	3	15	18	23	28
2	Immediately	10	10	20		30
3	4 hours	trace	trace	trace	10	15
4	8 hours	5	5	10	25	35
5	24 hours	2	2	4	30	35
6	48 hours	2	2	4	10	
7	48 hours	5	8	13	23	33
8	72 hours	10	3	13	23	

into the edema fluid of the burned area. In all three sections the animals were treated alike, i.e., each received 6 mgm. P. S. P. subcutaneously after the introduction of 50 cc. of water by stomach tube. The bladder, empty at the beginning of the experiment, was again emptied by pressure through

[1]Rather than write out the word phenolsulphonephthalein each time it is employed we have adopted the symbol P. S. P.

the abdominal wall at the end of one and two hours. In some instances the excretion for periods of 24 and 48 hours was also noted. The dye was estimated in the usual manner employing a Duboscq colorimeter. The results are detailed in table 1.

It will be noted that in the section relating to normal animals there is a considerable variation in the dye elimination, but that the extreme limits are not widely separated. In the animals with a burn, injected with the dye remote from the wound, in general the P. S. P. elimination approaches very closely to that of the normal animals. On the other hand, when the dye is injected in the early hours after a burn there is a distinct tendency for a "lag" in the elimination. (Compare animals, 1, 2, 3, with the remainder.) This period compares very well with that of the most rapid loss of fluid to the burned area and the interval during which the capillaries are most permeable, as judged by trypan blue entrance into the edema tissue. It would seem that P. S. P. elimination through the kidney is somewhat slowed by the great transport of fluid to the burned area. Of course, the need for water in the case of the burned animal is much greater than in the normal, and in the former instance undoubtedly a portion of the water supply is diverted to the wounded area from the usual urinary channel, thus creating a lag. Twenty-four hours after a burn when presumably the need for quick water mobilization has disappeared or at least has been diminished, the elimination of P. S. P. approaches more nearly the usual normal level.

An entirely different result is obtained when P. S. P. is injected into edema tissue of a burned animal. The dye excretion is very markedly diminished when injected in periods up to 72 hours after a burn. Even though the dye elimination is measured for a period of 48 hours, the total excretion usually does not exceed one-third of the quantity generally found in the urine of a normal animal 2 hours after injection of the dye. The evidence for a greatly decreased reabsorption in the burned area is very striking. If the dye is injected immediately after the burn is produced, the elimination seems somewhat better than at any later period observed. The least absorption occurs at the fourth hour, after which it improves somewhat but always remains at a very low level.

From these data, which quantitatively corroborate those with methylene blue and trypan blue, it is quite apparent that the absorption from the wounded area of a burned surface is markedly diminished. This is undoubtedly caused by the very greatly reduced permeability of whatever capillaries may remain still functional in the neighborhood of the wound.

4. THE ABSORPTION OF STRYCHNINE FROM THE EDEMA MASS. In order to test further the degree of absorption from the burned area use has been made of the well-known action of strychnine to produce convulsions and death after subcutaneous administration. The experiment was divided

TABLE 2

Strychnine injections

Normal rabbits—Dose of strychnine = 0.6 mgm. per kilogram

RABBIT NUMBER	WEIGHT	ONSET OF CONVULSIONS	REMARKS
	K.	*minutes*	
1	1.5	11	Dead in 25 minutes
2	1.3	11	Recovered
3	1.3	5	Dead in 10 minutes
4	1.5	10	Dead in 12 minutes
5	1.5	22	Recovered
6	1.4	10	Dead in 12 minutes
7	1.4	8	Dead in 14 minutes
8	1.5	13	Dead in 15 minutes
9	1.5	21	Recovered
10	1.0	15	Dead in 22 minutes
11	2.0	9	Recovered
12	1.7	7	Dead in 10 minutes

Burned rabbits—injection of strychnine remote from burn

RABBIT	WEIGHT	TIME OF STRYCHNINE INJECTION	ONSET OF CONVULSIONS	REMARKS
	K.	*after burn*	*minutes*	
1	1.5	Immediately	11	Recovered
2	2.0	1 hour	35	Recovered
3	1.7	2 hours	15	Recovered
4	1.7	4½ hours	11	Dead in 20 minutes
5	2.1	8 hours	28	Dead in 30 minutes
6	1.3	24 hours	10	Recovered
7	1.2	48 hours	5	Dead in 7 minutes
8	1.8	72 hours	7	Recovered

Burned rabbits—strychnine injected into burned area

1	0.6	Immediately	10	Dead in 12 minutes
2	1.1	Immediately	10	Dead next day
3	2.6	Immediately	10	Recovered
4	1.1	1 hour	No symptoms	Recovered
5	2.0	2 hours	No symptoms	Recovered
6	1.0	4 hours	No symptoms	Recovered
7	2.1	4 hours	No symptoms	Recovered
8	1.7	4½ hours	No symptoms	Recovered
9	1.9	8 hours	No symptoms	Recovered
10	1.0	24 hours	No symptoms	Recovered
11	1.2	48 hours	7	Recovered
12	1.2	72 hours	No symptoms	Recovered
13	1.2	72 hours	No symptoms	Recovered

TABLE 2—*Concluded*

Burned rabbits—strychnine injected at periphery of burned area

RABBIT	WEIGHT	TIME OF STRYCHNINE INJECTION	ONSET OF CONVULSIONS	REMARKS
	K.	*after burn*	*minutes*	
1	1.3	Immediately	No symptoms	Recovered
2	1.3	Immediately	No symptoms	Recovered
3	1.0	1 hour	11	Dead in 15 minutes
4	1.1	2 hours	No symptoms	Recovered
5	1.4	4 hours	No symptoms	Recovered
6	1.5	8 hours	No symptoms	Recovered
7	1.0	26 hours	9	Recovered
8	1.3	26 hours	No symptoms	Recovered
9	1.4	52 hours	No symptoms	Recovered
10	1.2	72 hours	No symptoms	Recovered
11	1.1	96 hours	No symptoms	Recovered

into four parts: 1, the determination of the number of deaths in normal animals after the injection of the usually accepted lethal dose for the rabbits, namely, 0.6 mgm. strychnine nitrate per kilo; 2, the injection of this dose subcutaneously into rabbits with burns in a region remote from the wounded area; 3, the injection of this dose of strychnine into the central portion of the burned area; 4, the injection of the same dose of strychnine in the periphery of the burned area. The results of this experiment are summarized in table 2.

Of 12 rabbits receiving the generally accepted lethal dose of strychnine, all went into convulsions in a period varying from 5 to 22 minutes, and 8 died within 25 minutes. The second group of animals received the strychnine injections at varying intervals after the burn, the drug being introduced in a region remote from the burned area, usually in the back. It is quite noteworthy that when rabbits 1, 2, and 3 were injected at intervals of a few minutes, 1 hour and 2 hours, respectively, after a burn convulsions were in order but recovery occurred in all three. At later periods convulsions were always induced and three out of five animals died. The production of convulsions in animals 1, 2, and 3, with ultimate recovery, may perhaps be interpreted to mean that in this early period after a burn, the absorption, even in a region remote from the burned area, has been slowed somewhat. It may well be that in this early interval water mobilization toward the burned area is so strong that reabsorption capacity in the opposite direction is overwhelmed, or else there is insufficient fluid available temporarily to care adequately for water movement in both directions simultaneously. Later, results more nearly approaching those of normal animals are obtained due perhaps to a tendency to reëstablishment of normal conditions of water exchange.

When strychnine is injected directly into the burned area striking results are obtained. If the injection is made immediately following the production of a burn convulsions are to be observed as with normal animals and death may or may not occur. This fact would lead one to believe that if the absorption of the poison depends upon the permeability of the capillary, a change in permeability, that is, decreased permeability at least, must require an appreciable time interval. In other words, the damage to the capillary by the application of heat is not immediate so far as absorption is concerned. It may well be that this latent period represents an interval which precedes the rapid exit of fluid from the vessels to the wounded area. The exact duration of this so-called latent period has not been exactly determined. Certain it is, however, that at the end of an hour there is a marked change which undoubtedly may be interpreted to mean an alteration in capillary permeability. At this time and thereafter, in general up to a period of 72 hours, the injection of strychnine into the burned area produces no more evidence of toxicity than a corresponding quantity of physiological salt solution. In other words, the absorption of the strychnine is so slow that there is never enough in the circulation at a given moment to produce characteristic symptoms. Undoubtedly some strychnine is continuously absorbed, just as it has been shown that P. S. P. is absorbed. It is, however, a quantitative relationship and one may perhaps assume that strychnine would not be absorbed at a rate exceeding that of P. S. P. The slow absorption of the strychnine is of course ascribed to an altered permeability of the capillary through injury by the heat applied.

If strychnine is injected into the periphery of the wound, one would naturally expect to find results varying with the degree of injury inflicted upon the capillaries with which the drug may come into contact. In this area there must be all degrees of injury to the capillary. As the table shows, strychnine is not rapidly absorbed even when injected into the periphery of the burned area. It is somewhat difficult to judge the line of demarcation between injured and normal tissue and it is quite probable that in nearly all instances the drug was administered to the injured area if one may judge by the results obtained. At any rate it is quite evident that even on the periphery absorption shows a marked decrease in rate.

If one summarizes the results obtained relative to changes in capillary permeability as measured by the experiments with the dyes, methylene blue, trypan blue, P. S. P. or those with strychnine, the outstanding features are 1, that there is an increased permeability of the capillaries in the burned area, so that substances pass more readily than normally from the capillary to the wounded area resulting in a significant edema; 2, that after a short latent period absorption from the burned area is much slower than it is under normal conditions. There is always some absorption but

the rate is decidedly decreased from which it may be inferred that the capillary permeability is diminished; 3 it is quite probable that capillary permeability may be increased in one direction and decreased in the opposite direction.

SUMMARY

From a study of the distribution of dyes, the inference previously stated, that blood concentration following a burn is due to fluid loss from the blood through increased capillary permeability, is substantiated. Under the conditions of a burn substances which normally do not pass the capillary wall are found in the edema fluid.

After a short latent period absorption from the burned area is much slower than it is under normal conditions. From this fact it may be concluded that capillary permeability in this direction is diminished. Not only is the rate of absorption diminished but substances like trypan blue, which readily pass from the blood to the injured area are not reabsorbed to a significant extent, if at all.

From these facts it becomes apparent that capillary permeability in one direction may exist simultaneously with decreased capillary permeability in the opposite direction.

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STUDIES ON THE MECHANISM OF WATER EXCHANGE IN THE ANIMAL ORGANISM

III. The Extent of Edema Fluid Formation Induced by a Superficial Burn

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In preceding papers (1) it has been stated that the water lost from the blood to the area injured by a superficial burn may assume proportions sufficiently great to lead to marked blood concentration with its consequent detrimental effects. Although in man the measurement of the water lost from the blood in this manner is incapable of accurate estimation for obvious reasons, in the experimental animal, owing to the peculiar formation of subcutaneous edema, the collection of the fluid is easily accomplished.

The purpose of the present paper is to indicate the quantity of water lost from the blood of the rabbit by the infliction of a superficial burn. Attention is particularly called to the extent of the response elicited from the relatively restricted area selected—approximately $\frac{1}{6}$ of the total surface area.

The methods employed in a previous paper (2) were utilized here and hence their repetition is unnecessary. In the application of the hot iron an attempt was made to obtain what is designated as a "light burn" as distinct from a "heavy burn." The surface area involved in both instances was approximately the same. The light burn differed from the heavy burn in that in the former the heat did not penetrate so deeply as in the case of the heavy burn. There can, of course, be no approach to accuracy in the degree, that is, the depth of heat penetration, of the burn obtained. The experiment was so arranged that the rabbits were killed at different intervals subsequent to the burn. Death was caused by stunning and bleeding. The edema fluid was collected by separating the edema mass from the burned skin by means of a sharp razor, and the water present was estimated by drying to constant weight.

[1] A portion of these data are taken from the dissertation presented to Yale University by Merl E. Fisk in candidacy for the degree of Doctor of Philosophy, June, 1929.

On reference to the table it is quite apparent that there is a significant quantity of water lost from the blood to the wounded area in a period as early as six hours after a burn and that fluid continues to accumulate up to a period of 24 to 36 hours; after which there is a general tendency for the quantity of fluid to be smaller. This latter fact is perhaps to be explained on the basis of reëstablished reabsorption from the wounded area. The figures for any period may be quite variable. For example, animals B64 and B8 although comparable so far as one may judge from appearances, behaved very differently to a presumably similar injury in that one yielded more than twice as much water as the other. Again B68, with a six hour burn, yielded three times as much water as B70 with an eight hour burn. This point is very clearly brought out in the twenty-two hour period of "heavy burns" where five animals (I, A, B, C, D) were employed. The variability here is noteworthy. Although there is no absolute quantitative response to the injury, in general the response is directly related to the time accumulation of fluid is allowed to continue. The figures for hemoglobin content of the blood show similar divergences. It should be emphasized that the figures for "maximum" are to be regarded as the maximum *observed*. Hemoglobin estimations were not made systematically and hence there has been no attempt to correlate the quantity of fluid lost to the wound with the figures for hemoglobin. With this understanding of the hemoglobin figures it becomes quite evident that loss of fluid to the wounded surface may or may not be accompanied by high blood concentration. In many instances the figures for hemoglobin at death show that the organism was able to compensate quite adequately for the water lost. It is true that in certain instances a high degree of blood concentration was attained but it was not maintained. The variability in both the hemoglobin figures and those for water lost to the wounded area are perhaps most easily explicable on the basis of differences in water reserve of the individual animals.

In general the response to a "heavy burn" is not necessarily greater than with a "light burn" although the largest losses are to be found with the "heavy burn." It would appear from this statement that the severity of a burn is not the determining factor in the production of edema fluid. It may be that when a certain degree of heat injury has been inflicted the maximum response is elicited; further degrees of heat intensity or penetration are not capable of producing greater responses of edema formation. Such a conception agrees well with the clinical observation that the extent of the area involved is of greater significance in the production of detrimental effects than the depth or severity of the burn.

The degree of water lost from the blood to the injured area is perhaps best interpreted in terms of its relation to the blood volume. The absolute figures for water necessarily bear a relationship to the weight of the

TABLE I

Water content of edema fluid induced by a burn

HOURS AFTER BURN	ANIMAL NUMBER	WEIGHT OF WATER IN EDEMA FLUID	EDEMA WATER AS PERCENTAGE OF TOTAL BLOOD VOLUME*	RELATIVE HEMOGLOBIN VALUES	
				Maximum	At death
A. Light burn					
		grams		*Normal = 100*	
6	B 61	2.98	6.3		137
	B 2	3.93	6.6	157	120
8	B 66	12.18	16.1	108	94
	B 3	9.83	13.9	100	98
10	B 62	6.94	13.8	136	112
	B 1	8.86	12.5	110	76
12	B 64	5.12	8.9		130
	B 8	13.32	21.9		120
16	B 10	10.72	20.5	115	102
	B 12	10.47	28.5		111
20	B 73	11.57	30.5		122
	B 7	8.34	21.9	110	91
26	B 74	14.29	26.0	153	144
	B 14	12.14	26.3	118	117
30	B 15	18.34	31.3	88	107
36	B 72	7.63	14.9	131	96
	B 33	17.22	23.7		121
40	B 34	8.67	20.4	156	129
44	B 81	3.68	8.0	129	100
48	B 18	8.04	8.0	132	112
50	B 60	5.21	8.7	159	123
56	B 20	11.72	20.5	152	95
62	B 45	13.19	19.8	128	101
78	B 22	2.16	3.2	113	117
B. Heavy burn					
6	B 68	18.89	19.5		123
8	B 70	5.78	6.1	140	108
10	B 76	5.39	10.8		138
12	B 88	21.28	19.4		142
16	B 65	13.65	23.2		140
22	I	12.06	46.6		
	A	27.12	63.0	123	100
	B	24.56	70.0	136	116
	C	13.65	40.0	122	120
	D[1]	6.55	25.0	131	121
26	O	4.16	20.0		
29	U	11.86	43.3		
32	B 86	16.89	17.8	146	110
34	B 79	11.48	15.8		102
36	B 82	14.64	21.5		115
38	B 85	17.58	18.5	158	118
40	B 78	18.04	24.7	146	118
43	F	9.93	36.6		
48	D	3.46	20.0		
52	M	2.12	12.0		

* Total blood volume—calculated as 5 per cent of body weight.

animals and since the weight varied in these experiments, the absolute figures are not strictly comparable. When this factor of body weight is taken into consideration as has been done by correlation of water loss to calculated blood volume the figures obtained are comparable. On this basis the greatest water loss occurs within the first 36 hours, the maximum observed being 70 per cent of the total blood volume. Such a water loss becomes of great importance in the water regulating mechanism of the body. It will be remembered that only approximately $\frac{1}{6}$ of the total surface area of the body was involved. If one assumes that a comparable loss occurs with multiples of this area it may be seen that the possible water loss may be very large and amply sufficient to account for some of the remarkably high figures for blood concentration in burns previously recorded (1). If it is permissible to apply these figures to man the results obtained are of interest. On the assumption that a man of 65 kilos has a blood volume of 5000 cc. and $\frac{1}{6}$ of the surface area were burned it would mean that 3500 cc. of water are poured out on the surface during the first 24 to 36 hours. Obviously such a water loss in addition to the ordinary maintenance losses indicates a need for replacement of fluid. It was upon such a conception that a method of treatment for extensive superficial burns was based (2). Clinical experience has demonstrated that when $\frac{1}{3}$ of the body surface has been burned a fatal outcome may result. Assuming that with a burn involving $\frac{1}{3}$ of the body surface twice as much water is lost as with a burn injuring $\frac{1}{6}$ of the body surface then it follows that as much if not more than 140 per cent of the blood volume may be lost to the injured area. This figure, approximately 7000 cc., may perhaps represent the limit to which the organism may give up its reserve water to maintain blood volume constant. If this unknown limit is exceeded one may deduce that the fatal outcome is largely influenced by the inability of the organism to furnish sufficient water to maintain the blood of a consistency compatible with adequate circulation. It is, of course, possible that with a burn involving $\frac{1}{3}$ of the body surface the water response may not be in the same ratio as with $\frac{1}{6}$ injury but is much smaller. Even though these figures may not represent the real conditions existent they do emphasize the fact that the water lost to the surface in an extensive superficial burn is a factor of great significance in the fate of the individual concerned, and they further indicate the need for fluid introduction in order to reëstablish efficient circulation.

SUMMARY

A superficial burn involving approximately $\frac{1}{6}$ of the total surface area of the rabbit induces within a period of 6 hours a significant loss of fluid from the blood, in the form of subcutaneous edema. The fluid reaches its maximum accumulation in the first 24 to 36 hours, after which there is a

tendency for it to diminish probably due to reëstablishment of the process of reabsorption.

It has been further demonstrated that under the experimental conditions the water loss to the blood may reach as much as 70 per cent of the total blood volume. It is probable that with larger areas injured by heat the water loss may be much greater.

The results of these experiments support our previous assertion that the fluid lost from the blood under the conditions of an extensive superficial burn may lead to an intense blood concentration with its subsequent detrimental effects, and that this loss of fluid from the blood demands replacement for the maintenance of an efficient circulation.

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STUDIES ON THE MECHANISM OF WATER EXCHANGE IN THE ANIMAL ORGANISM

IV. The Composition of Edema Fluid Resulting from a Superficial Burn

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Thus far in our consideration of edema fluid formed as a result of a superficial burn attention has been directed mainly to its water content and its significance as a water loss to the organism. Although this aspect of the problem is of the greatest importance edema fluid must be regarded as consisting of more than mere water or water and salts and consequently as an agency whereby the blood is deprived of other substances also. The peculiar behavior of the rabbit (already detailed) in producing subcutaneous edema instead of blisters affords an excellent opportunity for the collection of this fluid. Analyses of this fluid have been made and the results obtained form the basis of the present communication.

In order that adequate material might be available a sufficiently large number of comparable animals were subjected to a superficial burn under similar experimental conditions and were allowed to live for a definite interval. At death both the blood and edema fluid were collected. The individual sample of blood was allowed to clot and the serum was separated. Eventually all samples of serum for a given period were mixed, centrifuged and analyzed at once. The edema mass was first strained through a fine sieve and then pressed through cheesecloth. Like blood plasma, the edema fluid clots. The samples of edema fluid were finally united, centrifuged and subjected to analysis immediately.

The methods of analysis employed were as follows: Total nitrogen by the macro Kjeldahl; water content by drying to constant weight; non-protein nitrogen, precipitation of proteins by sodium tungstate sulphuric acid reagent with macro Kjeldahl determination of nitrogen of the filtrate; albumin and globulin by the method of Howe; reducing substance by Benedict's method; potassium method of Shohl and Bennett; sodium, method of Kramer and Gittleman; calcium, method of Kramer and Tisdall; magnesium, method of Denis; chlorides, Van Slyke method; phosphorus, method of Fiske and Subbarrow.

TABLE 1

The composition of edema fluid and its relation to blood serum

HOURS AFTER BURN		WATER	ASH	TOTAL NITROGEN	NON-PROTEIN NITROGEN	ALBUMIN NITROGEN	GLOBULIN NITROGEN	SODIUM	POTASSIUM	CALCIUM	MAGNESIUM	CHLORIDES	INORGANIC PHOSPHORUS	REDUCING SUBSTANCE	CHLORIDES AS SODIUM CHLORIDE	NUMBER OF ANIMALS USED	REMARKS
		per cent	*per cent*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*grams per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*grams per 100 cc.*		
12	Serum of burned animals			0.86	0.065	0.64	0.15	384	15.3	11.4	2.25	344	0.81	105	0.57	12	Tinged with blood
	Edema fluid			0.91	0.124	0.63	0.16	342	66.0	10.1	3.00	342	1.13	120	0.56		Tinged with blood
24	Serum of burned animals	93.4	0.84	0.83	0.068	0.58	0.18	353	15.0	10.1	2.75	317	0.66	115	0.52	6	Clear
	Edema fluid	94.7	0.95	0.69	0.094	0.46	0.14	380	30.7	9.3	3.50	360	1.00	99	0.59		Tinged with blood
36	Serum of burned animals	92.8	0.87	0.93	0.061	0.75	0.12	333	15.5	12.1	3.50	339	0.60	180	0.56	6	Tinged with blood
	Edema fluid	94.6	0.98	0.70	0.083	0.50	0.12	319	42.0	9.3	7.00	353	0.42	84	0.58		Tinged with blood
48	Serum of burned animals	93.1	0.87	0.88	0.068	0.69	0.12	368	15.0	11.7	1.49	321	0.66	108	0.53	12	Tinged with blood
	Edema fluid	93.9	0.97	0.90	0.121	0.60	0.18	322	48.5	8.9	3.59	399	0.93	77	0.66		Tinged with blood
	Normal Serum—non-fasting			0.98	0.076	0.61	0.29	315	16.7	12.4	3.48	319	0.78	143	0.45	3–6	
	Normal Serum—fasting overnight			1.04			0.24									4	
	Normal Serum—fasting two days			0.92			0.27									2	

The results are detailed in the table, where comparison is made between the edema fluid and the blood serum of the same animals. The outstanding feature of these figures is the striking similarity of composition of the two fluids. Indeed the composition of the two fluids so closely approximate each other that one is forced to the conclusion that this type of edema fluid must be regarded as plasma of the blood. It therefore becomes apparent that the passage of this fluid from the blood entails a serious loss upon the circulatory medium. It may be that a portion of the serious effects of an extensive superficial burn are due to this fact. For example, it is possible that when the plasma loss is sufficiently great so that a highly concentrated blood is maintained the difficulty experienced in reducing the concentration to a lower level compatible with life is associated with this loss of plasma. It is conceivable that the latent period of 24 to 36 hours, during which this difficulty of dilution of the blood is encountered, represents the interval essential for the reformation or restoration of the plasma elements. The possibility exists that the loss of protein from the plasma beyond a certain presumably definite but unknown limit diminishes the power of the plasma to retain water. In the figures quoted here such discrepancies are not apparent since there is little or no evidence of extensive depletion of the plasma proteins. It must be pointed out in this connection that in this investigation the extent of the burn was not sufficiently great to yield a maintained high blood concentration. Therefore for the present the query relative to the rôle of plasma proteins in water retention must remain unanswered. The maintenance of approximately normal blood plasma in spite of the very significant drain caused by the edema fluid is but another illustration of the remarkable capacity of the organism to maintain its composition.

Although it must be conceded that there is a marked resemblance between the composition of the edema fluid and the blood serum a comparison of the serum of these animals with the serum of normal animals reveals slight but significant differences. In general the figures for the serum of the normal animals are slightly higher in every respect tested, with the exception of sodium and chlorides. This would lead to the conclusion that the blood under the abnormal conditions is somewhat depleted of all its constituents but is still sufficiently normal to perform its required functions. Particularly striking is the difference for globulin nitrogen. At first it was thought that this high figure for globulin in serum of the normal non-fasting animal might be explicable upon the ground of adequate nutrition and that the lower figures for the burned animal's serum might be due to the fasting condition. As may be seen from the table, deprivation of food up to a period of two days does not materially alter the globulin value of the serum when compared to the non-fasting animal, hence this explanation is not feasible. It would appear therefore that of the protein

constituents of the plasma globulins are lost to a greater extent than are the albumins. Indeed the latter show little or no change.

The striking general resemblance between the composition of the serum of the burned animal and that of the edema fluid notably fails with certain constituents. For example, the figures for non-protein nitrogen are always higher in the edema fluid than they are in the serum, in some cases being twice as great. This fact must be of some significance but at present it is obscure. Further investigation concerning this factor is in progress. Also the figures for potassium, magnesium and inorganic phosphorus are usually much higher in the edema fluid than in the serum; this is particularly noteworthy for potassium. At first glance it would appear that the high potassium and phosphorus figures might be related to the fact that the edema fluid was always tinged with blood. That this is probably not the correct interpretation may be concluded from the fact that the serum whether clear and free from blood or tinged with blood to an extent approximating the color of the edema fluid yielded comparable figures for potassium. The other obvious possible explanation is that the loss of significant quantities of sodium and chlorides through the edema fluid may disarrange ionic conditions so that potassium enters the plasma from the red corpuscles to an unusual extent and finds its way into the edema fluid. Or it may be that the usual excretion of potassium is diverted from the urinary channel to find its way into the edema fluid. The latter possibility is under investigation. Whatever may be the final solution of the matter the fact remains that the potassium changes are quite noteworthy.

SUMMARY

The edema fluid produced by an extensive superficial burn so closely resembles the serum of the blood of the burned animal that it must be regarded as blood plasma.

Compared with the serum of a normal non-fasting animal both the serum of the burned animal and the edema fluid show a lower value for the globulin fraction. This decreased globulin content can not be ascribed to the nutritive condition of the animal but is probably related to the mechanism responsible for the formation of the edema fluid. This loss of protein may be associated with the difficulty experienced in causing water retention under conditions of highly concentrated blood.

Although in general the serum and the edema fluid of a burned animal bear a close resemblance there are marked divergences with certain constituents; non-protein nitrogen, potassium, magnesium and inorganic phosphorus are generally higher in the edema fluid than in the serum. This is most marked for non-protein nitrogen and for potassium. In the case of non-protein nitrogen the values may be doubled and potassium may be several times greater in the edema fluid than in the serum.

STUDIES OF THE MECHANISM OF WATER EXCHANGE IN THE ANIMAL ORGANISM

V. The Relationship of the Blood Chlorides to the Chlorides of Edema Fluid Produced by a Superficial Burn

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In 1923 (1) the following statement was made relative to a consideration of burns in man: "During health chlorids are remarkably constant, varying for the whole blood from 0.45 to 0.50 per cent calculated as sodium chlorid. It might, *a priori*, be expected that when rapid interchange of fluid occurs corresponding changes in salt balance would take place since it is to be assumed that when fluid leaves the tissues and blood in response to local inflammatory reactions the fluid poured on the surface is plasma or at least modified plasma. Certain it is that in war gas poisoning in the production of pulmonary edema the fluid entering the lungs has almost the same composition as that of the blood plasma. Moreover, under these circumstances which lead to highly concentrated blood the sodium chlorid of the blood decreases whereas the sodium chlorid of the lungs correspondingly increases. It is quite probable that applying similar reasoning to the process in burns whereby fluid is lost to the burned surface one should expect lowered chlorid content of the blood if the latter has reached a stage in fluid interchange in which normal water balance can no longer be maintained.

"Although the figures are in no sense complete, the data on sodium chlorid content of the blood plainly indicate that this salt played a significant rôle in fluid interchange at least during the initial stage of the injury. In general, when blood was highly concentrated, the sodium chlorid content was distinctly low and when the value for hemoglobin was not so greatly altered, the figures for sodium chlorid approach very closely those of the normal. Expressing it differently, almost without exception the sodium chlorid values were inversely as those for hemoglobin. On this basis the data for sodium chlorid divide the cases into two

[1] A portion of these data is taken from the dissertation presented to Yale University by Merl E. Fisk in candidacy for the degree of Doctor of Philosophy, June, 1929.

groups quite as sharply as the hemoglobin figures. It is, of course, quite probable that other constituents of the blood, especially the remaining inorganic components, play a distinct rôle in the water balance of the body. These possible changes were not investigated. It is quite apparent, however, that in the rapid interchange of fluid during the inflammatory stage of burns, sodium chlorid functions prominently. This observation tends to establish the hypothesis that in acute inflammatory processes the mechanism of fluid interchange in response to injury is of similar character whether the irritating agency is war gas or heat."

Somewhat later Davidson (2) confirmed these results for blood chlorides in burned patients.

Of the conceptions outlined in the quotations cited it has now been established experimentally that the fluid poured on the surface in response to a superficial burn partakes of the nature of plasma (3). The present paper is concerned with some aspects of the rôle of chlorides in the fluid interchange initiated by a superficial burn.

EXPERIMENTAL. The experiental procedures were identical with those outlined in previous papers. It should be emphasized that the burn inflicted injured approximately $\frac{1}{6}$ of the total body surface and that the experimental animals (rabbits) were denied food and water throughout the experimental period. The plan of the experiment was as follows: Three groups of animals were maintained under identical conditions. One group received a "light burn" (4), another, a "heavy burn" (4), and the third was anesthetized for the same period as the first two groups but was not burned. Blood chlorides were determined on all animals at the beginning and again at the end of the experiment, the interval of which varied as given in the table. With the burned animals the edema fluid was collected in the usual manner and the content of chloride determined.

A glance at the table demonstrates that almost without exception the blood chlorides, whether considered from the standpoint of whole blood or plasma, are not diminished under the experimental conditions. Indeed in most instances it would appear that they are increased. That this apparent increase is not related to the burn is shown by the fact that in the control unburned animals it is also existent. It is due therefore to some factor as yet unexplained. The degree of the burn is also apparently without influence upon the chloride of the blood, for the values with the "heavy burn" are similar to those of the "light burn." It would appear at first thought that the failure to diminish the chlorides of the blood under these experimental conditions fails to corroborate the results on man already quoted. Further consideration, however, shows that the present results simply confirm them. It was stated that "in general, when blood was highly concentrated, the sodium chlorid content was distinctly low, and when the value for hemoglobin was not so greatly

TABLE 1

The relationship of blood chlorides to the chlorides of edema fluid

HOURS AFTER BURN	ANIMAL NUMBER	MAXIMUM RELATIVE HEMOGLOBIN VALUES	BLOOD CHLORIDES				EDEMA FLUID		
			Normal		At death		Chlorides	Edema chlorides as percentage of total blood chlorides	Percentage of NaCl
			Whole blood	Plasma	Whole blood	Plasma			
A. Light burn									
		Normal = 100	*mgm./100 cc.*		*mgm./100 cc.*		*mgm./100 cc.*		
6	B 2	157	281	283	322	349	510	11	0.85
	B 61		258	296	258	335	330	8	0.55
8	B 3	100	280	318	340	403	300	15	0.50
	B 66	108	276	335	278	336	258	15	0.43
10	B 1	110	269	304	282	355	320	15	0.54
	B 62	136	253	327	266	300	282	15	0.47
12	B 8		283	358	371	419	210	16	0.35
	B 64		264	312	258	325	294	10	0.49
16	B 10	115	261	324	353	266	191	15	0.32
	B 12		291	324	379	374	230	22	0.38
20	B 7	110	285	298	382	358	350	27	0.58
	B 73		258	291	289	340	270	36	0.45
24	B 32		237	252	282	314	140	14	0.23
26	B 74	153	245	308	281	340	246	26	0.41
30	B 15	88	232	297	268	339	156	21	0.27
36	B 33		250	328	311	346		9	
	B 72	131	266	319	278	333	288	16	0.48
40	B 34	156	271	271	303	299	200	7	0.33
44	B 81	129	249	317	268	304	246	8	0.41
50	B 60	159	245	317	279	367	282	10	0.47
56	B 20	152	234	301	308	368	225	19	0.37
60	B 21	125	221	295	297	342	0		
64	B 38		287	259	353	326	0		
78	B 22	113	224	303	297	368	240	3	0.41
7 days	B 25	125	229	268	310	348	0		
B. Heavy burn									
6	B 68			346	249	346	282	18	0.47
8	B 70	140	251	357	244	312	372	9	0.62
10	B 76		255	310	274	340	258	11	0.43
12	B 88		264	324	257	317	144	10	0.24
16	B 65		266	315	295	346	243	21	0.40
32	B 86	146	262	375	266	328	264	18	0.44
36	B 82		253	233	295	350	294	25	0.49
40	B 78	146	275	325	274	304	252	22	0.42

TABLE 1—*Concluded*

HOURS AFTER ETHER	ANIMAL NUMBER	MAXIMUM RELATIVE HEMOGLOBIN VALUES	BLOOD CHLORIDES				EDEMA FLUID		
			Normal		At death		Chlorides	Edema chlorides as percentage of total blood chlorides	Percentage of NaCl
			Whole blood	Plasma	Whole blood	Plasma			
C. Controls									
		Normal = 100	*mgm./100 cc.*		*mgm./100 cc.*		*mgm./100 cc.*		
6	B 6	100	256	288	311	353			
10	B 5	100	282	309	291	362			
16	B 30	100	253	290	318	339			
22	B 29		253	292	332	356			
26	B 35	111	268	305	342	332			
30	B 36	107	268	308	314	355			
36	B 48		250	261	335	329			
40	B 39		274		355				
44	B 47	100	217	266	307	315			
48	B 46	111	248	245	297	313			

altered, the figures for sodium chlorid approach very closely those of the normal." If this statement is expressed differently it might read that sodium chloride of the blood does not show a diminution so long as the organism is capable of maintaining its water balance. When this mechanism fails the chlorides are lowered. The failure of this mechanism is indicated also by a maintained high blood concentration.

In the present work the extent of the burn was insufficient to induce a maintained high blood concentration. In many of these animals the hemoglobin figures reached temporary high levels but compensation was always attained. That compensation was possible is also attested to by the fact that none of these animals died because of the burn. The injured area was too small. It must, therefore, be apparent that the failure of the blood chlorides to be lowered is to be ascribed to the fact that the burn was not sufficiently extensive to deplete the chloride stores of the body. Just as the organism was capable of compensating for water it was equally capable of compensating for chloride.

Whether calculated as chloride or as sodium chloride it is also quite apparent that the edema fluid poured out partakes of the nature of the water-salt solution as it exists in the blood plasma. It is true that there are fluctuations in both directions but in general whether in serum or edema fluid the sodium chloride content approaches a concentration of 0.4 to 0.5 per cent solution.

Perhaps the most outstanding feature of this aspect of the burn problem relates to the quantity of sodium chloride that may be lost from the blood

without evidences of depletion of the blood chlorides. From the table it may be seen that when referred to the blood chloride content the sodium chloride present in the edema reveals very great differences, varying from 3 to 36 per cent. It appears quite noteworthy that more than $\frac{1}{3}$ of the chlorides of the blood may be lost from the tissue without apparent alteration in its composition, evidence again of the great ability of the organism to compensate for losses.

SUMMARY

Under the influence of a superficial burn blood chlorides show no diminution so long as blood concentration is maintained within normal limits. As demonstrated by previous work the chlorides are distinctly reduced in the blood when blood concentration is maintained at a high level.

The organism possesses a marked ability to compensate for chloride losses since as much as 36 per cent of the sodium chloride of the blood may be lost without causing alteration in the chloride content of the blood.

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(4) The meaning of these terms is explained in Paper III of this series.

STUDIES ON THE MECHANISM OF WATER EXCHANGE IN THE ANIMAL ORGANISM

VI. The Composition of Tissues under the Influence of a Superficial Burn

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The noteworthy outpouring of fluid from the blood to the injured area of a superficial burn leads to the query as to the ultimate source of the fluid. Presumably its direct origin is in the blood, and if the blood is to maintain its normal constancy of composition the substances lost must be replenished from a tissue or tissues. Water and salt are lost from the blood through this fluid in greatest quantities and inasmuch as these substances are easily mobilizable it should be possible to trace their exit from the tissues if the tissues are capable of yielding measurable quantities to the blood. That tissues must possess this capability may be gathered from earlier papers of this series in which it is shown that the organism without food or water possesses the ability to compensate water lost to the area injured by a burn to the extent of at least 70 per cent of the blood volume. That the degree to which water may be given up by the tissues is limited is attested to by the fact that ultimately compensation of a concentrated blood fails and the organism suffers from the effects of a failing circulation which leads to death.

In previous experience (1) with blood concentration maintained at a high level it has been impossible to find marked changes in the water content of the tissues even though the organism was greatly in need of water for maintenance of the circulation. This fact would lead one to the view that the tissues yield a certain limited quota of their water content with great readiness but that after this unknown but probably quite definite limit has been reached further attempts to cause the tissues to give up more are futile. It would appear that the organism clings tenaciously to the maintenance of this unknown water limit to conserve its constancy of composition even at the expense of the circulation.

That it is difficult to change the water content of tissues through fluid loss as a result of a burn receives support from the data herein presented.

Experimental. *Methods.* The investigation was so arranged that four groups of rabbits, maintained without food or water, were subjected to the

following procedure. The first group, 15 in number, served as controls and were merely anesthetized. The other three groups, comprising 27, 10 and 16 animals respectively were subjected to a burn under anesthesia and were allowed to live for varying periods. These periods were usually multiples of a four hour interval and generally extended to a period of 48 hours and in some instances to 72 hours. Three different degrees of burn were inflicted, a very light burn, a light burn and a heavy burn. These differed only in the depth of penetration of the heat. The area involved ($\frac{1}{6}$ of the body surface) remained constant in the three groups. The animals were bled to death, the tissues were rapidly excised, finely comminuted, sampled and analyzed for water, ash and chloride contents. Water content of the tissues was determined by drying duplicate samples in vacuo for four days, the temperature being gradually elevated from 50°C. to 104°C. After weighing the tissues were replaced in the oven for 24 hours and reweighed. The ash was estimated by treatment in an electric muffle at dull red heat for 48 hours or until a fluffy ash was obtained, white in most cases, black in the case of pigmented tissues like the liver and skin. The degree of heat was sufficient to volatilize all the chlorides present. The method of Van Slyke was employed for the estimation of tissue chlorides.

The composition of the tissues after a burn. In table 1 is given the average composition of tissues of animals subjected to the procedure outlined above. In order to conserve space only the average figures are presented. These, however, accurately represent the individual variations observed in the animals of a single group or of different groups. The variations are very slight even though the different groups represented are quite diverse so far as the number of animals is concerned. The figures remain unchanged whether a group of 10 or a group of 27 is considered. The striking feature of these figures is that under the markedly different conditions of water loss as represented by the different degrees of injury from the heat the composition of tissues remains unchanged and does not diverge from the composition of the tissues of the normal animals not subjected to the extraordinary water loss entailed by an extensive burn. The composition of the different tissues varies only slightly, especially with respect to the water and ash contents. The chloride content is much more variable, the liver being particularly low in this respect.

In consideration of these figures it should be pointed out that the extent of burn in these cases was not sufficient to lead to death, and although the water loss to the body was significant, approaching 30 per cent of the blood volume, compensation of the blood volume, as indicated by relative hemoglobin content, was always attained. Nevertheless, the results of the experiment demonstrate with marked clarity the extreme difficulty of changing tissue composition by loss of fluid from the blood.

Changes in composition of burned tissues. In these experiments the tissues involved in a burn are the skin and the abdominal muscle. In tables 2 and 3 are given details relative to the changes in the skin and abdominal muscle after varying degrees of a burn. These data are derived from the same animals considered in table 1. In each 12 hour period several animals varying from 4 to 6 are included.

TABLE 1

The average composition of tissues under the influence of a burn

	ANIMALS		WATER CONTENT	ASH CONTENT	CHLORIDE CONTENT
	Description	Number			
			per cent wet tissue	*per cent wet tissue*	*mgm./100 grams wet tissue*
Lung	Normal	15	80	1.32	175
	Very light burn	27	79	1.38	175
	Light burn	10	79	1.42	180
	Heavy burn	16	79	1.32	162
Liver	Normal	15	74	1.42	83
	Very light burn	27	75	1.52	86
	Light burn	10	75	1.42	71
	Heavy burn	16	74	1.45	71
Kidney	Normal	15	78	1.38	178
	Very light burn	27	78	1.37	183
	Light burn	10	78	1.39	154
	Heavy burn	16	77	1.35	175
Small intestine	Normal	15	81.	1.35	113
	Very light burn	27	81	1.37	121
	Light burn	10	81	1.33	128
	Heavy burn	16	81	1.41	119
Spleen	Normal	15	78	1.50	
	Very light burn	27	79	1.50	
	Light burn	10	79	1.59	
	Heavy burn	16	78	1.54	

If the data relative to the burned skin (table 2) are considered it may be observed that noteworthy changes in composition occur. In the first place it is quite evident that the unburned skin of a burned animal varies little in water content from the skin of the normal rabbit. There is, it is true, at times a suggestion that the water content is somewhat lower, carrying with it the possibility that the unburned skin has contributed water from its store. The differences are quite variable, however, and are

not sufficiently significant to establish such a view. When the burned skin is considered it is quite evident that the water content with a very light burn is increased without doubt. On the other hand when the severity of the burn is increased there is a distinct tendency for the water content to be diminished. With the very light burn the change in water con-

TABLE 2

Changes in the composition of the skin after a superficial burn

	SKIN OF NORMAL ANIMAL	UNBURNED SKIN OF BURNED ANIMAL			BURNED SKIN		
		Very light burn	Light burn	Heavy burn	Very light burn	Light burn	Heavy burn
A. Water content (expressed as percentage of wet tissue)							
1st 12 hour period	77	73	70	70	84	69	62
2nd 12 hour period	72	69	74	70	81	58	71
3rd 12 hour period	72	69	68	73	81	70	70
4th 12 hour period	69	74	69	72	79	70	53
5th 12 hour period	71	72			77		
6th 12 hour period	72	71			80		
At end of 7 days				70			72
B. Ash content (expressed as percentage of wet tissue)							
1st 12 hour period	0.80	0.76	0.57	0.74	0.79	0.80	1.09
2nd 12 hour period	0.72	0.99	0.82	0.63	0.98	1.20	
3rd 12 hour period	0.78	0.73	0.70	0.70	1.01	1.02	1.11
4th 12 hour period	0.73	0.80	0.62	0.81	1.13	1.17	1.16
5th 12 hour period	0.74	0.93			1.25		
6th 12 hour period		0.73			1.08		
At end of 7 days				0.73			1.24
C. Chloride content (expressed as milligrams per 100 grams wet tissue)							
1st 12 hour period		174	173	201	238	263	214
2nd 12 hour period	158	208	187	179	298	243	251
3rd 12 hour period	178	180	161	229	270	289	238
4th 12 hour period	171	190	229	232	267	285	244
5th 12 hour period	174	196			251		
6th 12 hour period	177	166			195		
At end of 7 days				229			135

tent undoubtedly represents the well-known edematous condition of the skin under a very light burn whereas the drier condition of the light and heavy burn represents more nearly the parchment-like condition seen with these types of burns.

With the very light burn the ash content of the unburned skin shows a

tendency to increase and this tendency is further emphasized with this type of burn involving the burned skin. With the light and heavy burn the ash content of the unburned skin is either normal or tends to decrease. The ash content of the burned skin with both the light and heavy burn is distinctly increased especially after the first 12-hour period. These in-

TABLE 3

Changes in the composition of muscle after a superficial burn

	MUSCLE OF NORMAL ANIMAL		UNBURNED MUSCLE (LEG) OF BURNED ANIMAL			BURNED MUSCLE (ABDOMEN)		
	Leg	Abdomen	Very light burn	Light burn	Heavy burn	Very light burn	Light burn	Heavy burn
A. Water content (expressed as percentage of wet tissue)								
1st 12 hour period	79	77	78	78	77	83	87	86
2nd 12 hour period	78	78	78	78	79	84	84	85
3rd 12 hour period	79	79	78	77	77	83	86	86
4th 12 hour period	79	78	78	77	79	86	82	83
5th 12 hour period			78			84		
6th 12 hour period			77			80		
At end of 7 days					79			79
B. Ash content (expressed as percentage of wet tissue)								
1st 12 hour period	1.19	1.07	1.26	1.06	1.37	1.03	0.91	0.99
2nd 12 hour period	1.23	1.03	1.37	1.34	1.09	1.03	1.04	0.83
3rd 12 hour period	1.20	0.96	1.18	1.57	1.37	0.95	0.92	0.90
4th 12 hour period	1.28	1.00	1.13	1.52	1.41	1.03	1.03	1.13
5th 12 hour period			1.39			1.33		
6th 12 hour period			1.03			1.31		
At end of 7 days					1.20			1.16
C. Chloride content (expressed as milligrams per 100 grams wet tissue)								
1st 12 hour period	39	62	42	41	35	231	212	237
2nd 12 hour period	49	62	63	54	39	228	252	232
3rd 12 hour period	36	61	42	44	44	198	243	266
4th 12 hour period	39	69	42	40	45	218	237	240
5th 12 hour period			40			216		
6th 12 hour period			40			218		
At end of 7 days					47			247

creases of ash content cannot be ascribed to chloride content since by the method of ash determination, the chlorides were completely volatilized. The increase must therefore be due to other salts.

That the chlorides play a distinct rôle in interchange between the blood and skin is evidenced by the figures for chlorides in table 2. Even in the

unburned skin it is quite apparent that with each type of burn there is a noticeable tendency for the chlorides to increase. The augmented chloride content is very striking in the case of the burned skin and as may be seen from the table bears no constant relationship to the water content of the skin. The chlorides apparently play a prominent rôle in the manifest attempt of the body to alleviate the effects of injury caused by the heat. Another possibility exists relative to the increased chloride content of the skin. Comparison of the chloride content of the burned skin with the chloride content of the whole blood shows a striking similarity. In general the figures are almost equivalent. It is therefore possible that these high figures for the chlorides of the skin represent blood that has been trapped in the burned area. Blood vessels are undoubtedly injured even with the slightest burn and although on inspection most of the large vessels appear patent and functioning it is quite possible that a considerable portion of blood remains in a condition of stasis in the injured area. One of the initial responses to the injury is a flushing of the part with blood in which according to this view the entire skin must partake since even the uninjured skin of the burned animal is higher in chlorides than is the skin of the normal animal. That this is not a transitory condition may be seen from the table since it continues up to a period of at least 60 hours after a burn. Whether such a condition is to be regarded as a compensatory reaction or as a loss of coördination of function remains uncertain. In any event the skin of the burned animal contains an unusually large quantity of chlorides in both the unburned and burned areas. If the matter is considered from the standpoint of sodium chloride in solution in the water present in the skin the following percentages are obtained:

Percentage of sodium chloride in water contained in skin

CHARACTER OF SKIN		PERCENTAGE OF NaCl
Normal		0.30
Unburned skin of burned animal	Very light burn	0.35
	Light burn	0.37
	Heavy burn	0.41
Burned skin	Very light burn	0.45
	Light burn	0.56
	Heavy burn	0.51

In a previous paper (2) it has been shown that the serum of a normal rabbit contains 0.45 per cent NaCl and the serum of the burned animal 0.54 per cent NaCl.

From the figures given above it is quite apparent that the skin of the normal animal contains a lower percentage of sodium chloride than does the normal serum and that under the influence of a burn even the unburned skin tends to increase in its relative content of sodium chloride. This increase is much more marked in the case of the burned skin and approaches closely the percentage of sodium chloride content of the serum of the burned animal. From these considerations it must be evident that sodium chloride functions in some important manner in the changes occurring in the injured area. It would seem that this function can hardly be regarded as a passive one because of the variability of the chloride content and its apparent direct relationship to the severity of the burn. It is a function in which the uninjured skin of the burned animal also partakes.

In table 3 are given data relative to changes in composition of the muscle of burned animals. In these experiments the only muscle involved in the burn is the abdominal muscle but for the sake of comparison the uninjured muscle of the leg has been analyzed also. From the figures it is quite apparent that although the two kinds of normal muscle contain approximately the same amount of water the ash and chloride contents are essentially different. The ash content of the leg muscle is appreciably higher than that of the abdominal muscle in spite of the fact that the abdominal muscle is significantly richer in sodium chloride content.

There are no figures available for the unburned abdominal muscle of the burned animal but it is quite certain from the figures in table 3 that the leg muscle under these circumstances changes little if at all. In this respect therefore the musculature of the body differs from the skin since with the latter an extensive injury of one part affects all parts to a certain degree.

With the injured portion of the abdominal muscle it is quite evident that changes occur quite analogous to those of the injured skin. With all types of burns inflicted the water content of the tissue is markedly increased which would be expected from the obviously edematous condition of the injured tissue. On the other hand, the ash content shows little or no modification. As with the skin, the injured muscle tissue is very rich in sodium chloride, the absolute quantities approximating those of the skin. When, however, calculation is made of the quantity of sodium chloride relative to the water content it is found that the figures so obtained while much greater than under normal circumstances are of smaller proportions than obtain for similar figures for the injured skin. Nevertheless it must be conceded that the injured muscle attracts to itself much greater quantities of chlorides than does the skin since in the former case the chlorides are three-fold the normal values, whereas in the case of the skin the chlorides are less than double the normal value. The same fact is indicated in the calculations for sodium chloride percentages in the water of muscle and skin.

Percentage of sodium chloride in water contained in muscle

CHARACTER OF MUSCLE		PERCENTAGE OF NaCl
Normal	Abdominal	0.11
	Leg	0.07
Unburned leg	Very light burn	0.08
	Light burn	0.08
	Heavy burn	0.07
Burned abdominal muscle	Very light burn	0.38
	Light burn	0.38
	Heavy burn	0.39

These considerations make it evident that the water, sodium chloride and other salts lost to the skin and muscle from the blood must be regarded as losses for a period of at least three days. That these losses may perhaps in part be regarded as permanent losses is suggested by analyses made at a period of 7 days (see tables 2 and 3). At this period the water content of the skin is within the usual range for the type of burn inflicted and the ash content is even slightly greater than at earlier periods. With the unburned skin the chlorides are still high but in the burned skin there is evidence of a decrease which probably indicates absorption of the chlorides.

With the muscle the water content has regained the normal level with little or no change in the ash and chloride contents. Absorption if it occurs at all is very slight. These indications of little or slow absorption agree well with the conclusions drawn in an earlier paper (3) derived from a different type of experiment.

SUMMARY

Under the experimental conditions the significant fluid loss induced by an extensive superficial burn does not alter the composition of the tissues with respect to water, ash and chloride contents. The degree to which these substances may be lost to the blood without essential modification of the tissues is noteworthy and presumably the composition of the tissues is conserved in preference to that of the blood.

When a portion of the skin is injured by a burn the entire skin becomes involved with respect to its sodium chloride content which is markedly increased. There is the same tendency for the ash content. In general the water content is not augmented. In the injured area the sodium chloride content is greatly increased whether the water content is or is not increased; the latter is dependent upon the type of burn inflicted. It is therefore apparent that the chlorides play a prominent rôle in the interchange between the blood and the injured tissue. In muscle tissue injured

by a burn the water content is markedly increased but the ash content varies little from the normal. On the other hand, the chloride content is increased to much greater proportions than is true for the skin.

The deposition of chloride in the area injured by a burn cannot be regarded as transitory since it remains unchanged for a period of three days and in the case of the muscle is still high at the end of seven days. At this period the burned skin has a low chloride value indicating absorption.

The data of this investigation add confirmatory evidence that the reabsorption of materials from the area injured by a burn is a very slow process.

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STUDIES ON THE MECHANISM OF WATER EXCHANGE IN THE ANIMAL ORGANISM

VII. An Investigation of Dehydration Produced by Various Means

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The determination of the water content of the animal organism represented one of the earliest approaches to an understanding of the mechanism of vital processes. The constancy of the total water content of the bodies of animals of different species, the regularity of its percentage distribution to the various organs in all species, the inability of the organism to survive water deprivation for a considerable period of time, and the speed with which any excess of fluid introduced into the body is removed, all indicate not only that water is essential for life but also that the vital functions hinge upon a delicate adjustment in the distribution of water in the organism. Despite the early recognition of these facts, it was not until the last decade that changes in the water content of the organism—more particularly in the direction of dehydration—were recognized as factors underlying many pathological disturbances. Such apparently different clinical conditions as Asiatic cholera, infant diarrheas, intestinal obstruction, influenza, war-gas poisoning and extensive superficial burns exhibit as a common symptom a marked concentration of the blood; all show unmistakable improvement when the concentration is relieved by forcing fluids; all proceed to fatality if the concentration is not reduced. Underhill (1) has emphasized the fact that a concentrated blood means "a failing circulation, an inefficient oxygen carrier, oxygen starvation of the tissues, a fall of temperature, and finally a suspension of vital activities."

It is the generally accepted theory that the tissues act as reservoirs to maintain the water content of the blood at a constant level through the removal of excess fluid from the blood in hydremia and the release of fluid to the blood in anhydremia. Much work has been carried through to determine the ability of the tissues to store excessive amounts of water

[1] A portion of these data is taken from the dissertation presented to Yale University by Merl E. Fisk in candidacy for the degree of Doctor of Philosophy, June 1929.

introduced into the body, but very few attempts have been made to discover to what degree the tissues can yield their water to a dehydrated blood. The fact that a degree of concentration of the blood incompatible with life may persist suggests either that the tissues cannot yield their "essential" water or that the release of even a small part of their essential water entails such a disruption of the protoplasmic equilibria that death ensues. The present work represents an effort to determine the ability of the tissues to yield their essential water.

The amount of water that can be mobilized for any one function probably depends upon the quantity of available, "free" or "interstitial" water present in the tissues. The "free" water is presumably liquid at ordinary temperatures, chemically uncombined and therefore capable of being shifted about, via the blood stream. It is contrasted with the "bound" or "essential" water which exists in a non-liquid form as hydrates of salts, carbohydrates and proteins, especially associated with colloidal systems. The bound water of the tissues can be increased or decreased by a variety of conditions. Any change in the bound water must of necessity increase or decrease the free water, but that the extent to which the bound water may be released is distinctly limited has been manifested in many experimental and pathological conditions.

Whereas previous workers have directed their attention almost exclusively to the changes in the composition of the blood, the present work is focussed mainly upon the changes in the tissues. In view of this fact and the existence of adequate reviews on the various phases, the very extensive literature on water metabolism will be omitted here.

EXPERIMENTAL. The investigation is divisible into several parts, dehydration being produced by 1, food and water deprivation; 2, food and water deprivation combined with the influence of phlorhizin; 3, hemorrhage; 4, the injection of hypertonic salt solution; 5, the injection of hypertonic glucose solution.

Methods. The experimental animals were full-grown rabbits. Samples of blood for the normal values were drawn from the marginal ear vein; later samples were sometimes taken from a larger vein or from an artery. Unless otherwise stated food and water were removed 6 to 8 hours before the experiment and were not given during its entire course. At the end of the experiment, the animal was stunned by a blow upon the neck and exsanguinated by opening the large vessels.

The work consisted in the determination of the hemoglobin concentration throughout the experiment, and of the total solids, whole blood and serum chlorides at the beginning and end of the experiment. The hemoglobin concentration was estimated by the Newcomer method, results being reported on the basis of the normal reading as 100. The blood chlorides were determined by the method of Van Slyke. For the total solids 1 cc. of blood was dried at 104°C. to constant weight.

The tissue work involved the determination of the water, ash and chloride contents, the methods employed being those outlined in paper VI of this series.

TABLE 1

The average composition of tissues under the influence of food and water deprivation

TISSUES	ANIMALS	NUMBER	WATER CONTENT	ASH CONTENT	CHLORIDE CONTENT
			per cent wet tissue	*per cent wet tissue*	*mgm./100 grams wet tissue*
Lung	1*	9	80	1.22	168
	2*	2	78	1.11	181
	3*	1	78	1.31	144
Liver	1*	9	74	1.39	69
	2*	2	73	1.43	72
	3*	1	75	1.20	80
Skin	1*	9	75	0.75	172
	2*	2	71	0.71	176
	3*	1	71	0.71	152
Abdominal muscle	1*	9	78	1.24	53
	2*	2	75	1.15	57
	3*	1	78	1.03	72
Leg muscle	1*	9	80	1.20	46
	2*	2	75	1.21	44
	3*	1	76	1.19	32
Kidney	1*	9	78	1.32	148
	2*	2	77	1.30	137
	3*	1	73	1.20	160
Spleen	1*	9	79	1.50	
	2*	2	78	1.63	
	3*	1	79	1.56	
Small intestine	1*	9	81	1.35	113
	2*	2			
	3*	1	80	1.10	117

1* Normal.
2* 12 days' water and food deprivation.
3* 4 days' water and food deprivation.

1. Dehydration produced by food and water deprivation. In these experiments three large full-grown animals were deprived of food and water to the limit of their endurance; one developed an alarming weakness on the fourth day and was killed; the other two survived until the twelfth day.

A comparison of the average water content of the tissues of the animals that survived 12 days with the average for the 9 normal animals reveals a small but definite tendency toward a decreased water content in most of the tissues, with changes especially marked in the case of the skin, muscles and lungs (see table 1). The skin exhibits a decrease in water content of 4 per cent, about the same decrease in ash and little change in the chloride content. The lungs have lost 2 per cent in water while the abdominal muscle and the leg muscle have suffered a loss of 3 and 5 per cent, respectively. With the animals with a 4 days' water and food deprivation the changes follow the same general tendency but are less constant.

The exact cause of the death from starvation is not known. It is presumed that the complete exhaustion of the reserve foodstuffs precipitates

TABLE 2

Blood changes produced by phlorhizin injections

TOTAL NUMBER OF DAYS WITHOUT FOOD OR H_2O	RABBIT NUMBER	WEIGHT	RELATIVE HEMOGLOBIN VALUES		BLOOD TOTAL SOLIDS		BLOOD CHLORIDES				VOLUME OF FLUID INJECTED	URINE VOLUME
							Normal		At death			
			Maximum	At death	Normal	At death	Whole blood	Plasma	Whole blood	Plasma		
		K.			*grams/100 cc.*		*mgm. of Cl/100 cc.*				*cc.*	*cc.*
8	019C	2.1	112	92	16.6	17.4	308		321		31.5	204
9	019D	2.3	109	102	18.3	17.0	293		321		34.5	120
8	F7	2.0	102	102	15.6	20.3	288	344	305	363	30.1	155
8	F8	2.2	103	96	17.0	19.8	303	355	295	348	33.3	160
8	F9	1.8	123	125	19.0	21.7	253	336	286	391	27.2	130

N. B.: Phlorhizin in doses of 0.25 gram/kilo was injected on 5 consecutive days into 019C and 019D, and on 3 days into the others.

an extensive catabolism of the tissues which entails such a disorganization of the various equilibria that the entity of the organism is destroyed. The autolysis of the tissues is associated with the liberation of the water held in combination with the various protoplasmic elements. This water is not completely free, however, for it tends to remain in the tissues furnishing the source of the increased water content found in starved animals which have access to water. So far as we are aware, the present work contains the first analysis of tissues from animals subjected to extreme fasting combined with complete water deprivation. Under these experimental conditions most of the tissues decrease in water content, indicating that as the water is freed by autolysis it is drawn into the blood stream to meet the needs of metabolism.

A rough calculation of the water lost from these animals, using their

average weight and the average value for the percentage of the total weight represented by each organ as given by Skelton (2) gives a loss of water of approximately 37 grams or about 33 per cent of the total blood volume. The amount of water yielded by all the tissues other than the skin and muscles amounts to 2 or 3 grams. The water reserves of the body are, therefore, normally stored in the skin and muscle, the other organs carrying much less reserve.

TABLE 3

The average composition of tissues under the combined influence of food and water deprivation and phlorhizin

TISSUES	ANIMALS	NUMBER	WATER CONTENT	ASH CONTENT	CHLORIDE CONTENT
			per cent wet tissue	*per cent wet tissue*	*mgm./100 grams wet tissue*
Lung	Normal	9	80	1.22	168
	Experimental	5	79	1.35	163
Liver	Normal	9	74	1.39	69
	Experimental	5	73	1.40	75
Skin	Normal	9	75	0.75	172
	Experimental	5	69	0.69	138
Abdominal muscle	Normal	9	78	1.24	53
	Experimental	5	76	1.13	51
Leg muscle	Normal	9	80	1.20	46
	Experimental	5	77	1.27	45
Kidney	Normal	9	78	1.32	148
	Experimental	5	78	1.39	141
Spleen	Normal	9	79	1.50	
	Experimental	5	79	1.66	
Small intestine	Normal	9	81	1.35	113
	Experimental	5	81	1.40	131

Conclusion. Food and water deprivation over an extended period results in small but definite decreases in the water content of the tissues. The skin and muscle are probably the great water reservoirs of the body.

2. Dehydration produced by a combination of food and water deprivation and the influence of phlorhizin. Five large animals were subjected to an eight-day period of food and water deprivation with phlorhizin injections on three or five consecutive days. Since phlorhizin is known to increase

tissue metabolism through a persistent glycosuria combined with mild diuresis and to produce an hydremia (at least in animals given water) (3), it was hoped that its use would magnify the changes taking place within the tissues in food and water deprivation. The animals of this experiment excreted a volume of urine equivalent to four to five times the total volume of fluid received in the three injections (table 3). The hemoglobin content fluctuates near the normal with a tendency toward an hydremia. However, the total solids at death show a decided increase over the solids in the blood drawn before the fasting period in four of the five animals. The increases amount to 4, 30, 16 and 14 per cent of the normal values. In this experiment, it is quite probable that the total solid changes are a better guide to the situation in the blood than is the hemoglobin content. It is not at all unlikely that the experimental conditions are productive of destruction of the red cells, and, hence, the hemoglobin content under these circumstances gives a perverted picture. In all cases there is a slight increase in the chlorides of the whole blood and of the serum.

The tissues (table 3) of these animals in general contain less water than under normal circumstances but more than the tissues of animals deprived of water and food to the limit of endurance. The skin and liver alone have a water content as low as the tissues of the exhausted animals. The skin also suffers a decrease in ash and chlorides, and is, perhaps, the source of the salts excreted in the urine.

The increased concentration of the blood of the phlorhizinized animals bears testimony to an embarrassment of the water economy even though the tissues are by no means destitute of water. It is true that the skin and liver have suffered as great a percentage decrease in water content as the same organs in the exhausted animals, but the muscles could still yield approximately 50 grams of water before reaching the low level of fatal water deprivation, and the vital organs show only slight decreases in their water content. When there is water enough available to more than offset the degree of concentration of the blood (14 to 30 per cent) that has developed, it is unlikely that the organism would permit the blood to remain an inefficient oxygen carrier unless the release of the remaining water involved difficulties equally or more serious than a concentrated blood. It is quite probable, therefore, that a concentrated blood is a less serious condition than the release of essential water from the tissues.

Conclusion. The addition of the influence of phlorhizin to food and water deprivation induces release of water in the tissues to no greater extent than food and water deprivation alone for a somewhat longer period. As in the latter instance, the skin bears the greatest loss in water, ash, and chloride content.

3. Dehydration produced by hemorrhage. The six animals employed in this work were divided into two equal groups. In one, the animals were

bled at once; in the other, bleeding occurred after a three-day deprivation of food and water. (See table 4.) From the data, it is apparent that animals deprived of food and water for three days restore their blood volume as efficiently but a trifle more slowly than normal animals. The restoration is complete within four hours and is followed by a period of over-dilution which may last as long as two days in a dehydrated animal five days without water. The total solids of the blood are decreased in every case, but are two to three times higher than they would be if there were no replacement with the same degree of dilution. The chlorides, however, are increased 20 per cent or more. Hence, the diluting fluid

TABLE 4

Blood changes after extensive hemorrhage

NUMBER OF ANIMAL	WEIGHT	CONDITION	TOTAL BLOOD VOLUME	VOLUME OF HEMORRHAGE	PER CENT OF TOTAL BLOOD VOLUME REMOVED	HOURS ELAPSING BEFORE DEATH	RELATIVE HEMOGLOBIN VALUES AT DEATH	RELATIVE HEMOGLOBIN VALUES IF ORIGINAL BLOOD VOLUME HAD BEEN RESTORED	PER CENT RESTORATION	TOTAL BLOOD SOLIDS			TOTAL BLOOD CHLORIDES			
													Normal		At death	
										Normal	At death	Per cent decrease	Whole blood	Plasma	Whole blood	Plasma
	K.		cc.	cc.						grams/100 gms.			mgm. of chloride/ 100 cc.			
F1	1.6	Normal	80	31	44	6	47	61	130	18.0	15.1	21	257	321	311	362
F3	1.6	Normal	80	29	40	4	61	64	103	18.3	15.2	15	248	308	303	367
F6	1.6	Normal	80	35	45	1	97	56	58	19.3	11.2	21	229	253	336	353
I1	1.8	Fasted with-	90	40	38	1	93	56	60	17.3	13.6	16	268	303	303	337
I2	2.0	out water	100	40	36	3	71	60	85	17.5	14.8	17	254	303	307	351
F2	2.0	for 3 days	100	45	43	48	32	55	171	17.4	13.7	42	272	332	331	353

must be relatively poor in solids but not in chlorides. There can be no question in this instance as to the source of the diluting fluid, for no water was given.

The tissues (see table 5) of the normal rabbits after hemorrhage show no significant loss of water except the skin. Hemorrhage after a three-day period of food and water deprivation causes an even greater depletion of water in the skin. The muscles of the leg have also suffered a loss of water. The ash and chloride values fail to exhibit any alteration beyond the limits of normal variation. The results agree well with those of Skelton (4) in the essential facts that the greater part of the water comes from the skin

and the muscles. However, Skelton reported in normal animals a loss of water per 100 grams of tissue two to three times greater in the liver than in the skin; while in animals 32 to 72 hours without water the liver lost about

TABLE 5

The average composition of tissues in dehydration produced by hemorrhages

TISSUES	ANIMALS	ANIMAL NUMBER	WATER CONTENT	ASH CONTENT	CONTENT CHLORIDE
			per cent wet tissue	*per cent wet tissue*	*mgm./100 grams wet tissue*
Lung	1*	9	80	1.22	168
	2*	3	80	1.31	133
	3*	3	79	1.35	150
Liver	1*	9	74	1.39	69
	2*	3	74	1.37	74
	3*	3	74	1.43	81
Skin	1*	9	75	0.75	172
	2*	3	71	0.68	158
	3*	3	66	0.63	170
Abdominal muscle	1*	9	78	1.24	53
	2*	3	80	1.07	60
	3*	3	77	1.30	49
Leg muscle	1*	9	80	1.20	46
	2*	3	80	1.21	51
	3*	3	77	1.14	49
Kidney	1*	9	78	1.32	148
	2*	3	78	1.41	154
	3*	3	79	1.25	158
Small intestine	1*	9	81	1.35	113
	2*	3	81	1.35	111
	3*	3	80	1.25	128
Spleen	1*	9	79	1.50	
	2*	3	79	1.37	
	3*	3	79	1.57	

1* Normal—without hemorrhage.
2* Normal—with hemorrhage.
3* 3 days' food and water deprivation with hemorrhage.

as much per 100 grams as the skin. This great loss of water from the liver was not found in the present work; in fact, the liver showed no loss of water. Skelton took samples for analysis from the living animal, thereby including

large amounts of blood which he tried to remove by drying with filter paper. In this case, the loss of fluid from the liver must probably be attributed to a loss of blood rather than of water. Although the two methods of sampling apparently yield essentially the same results, the method of taking samples during life may give a less accurate picture and may involve a serious error, as in this instance.

Conclusion. Hemorrhage in normal animals or in animals previously subjected to food and water deprivation induces a condition of blood dilution.

Under these circumstances, water is lost from the skin alone (in normal animals) or from the skin and muscles (in animals without food and water). The water loss is greater following hemorrhage when the animal has been deprived of food and water.

TABLE 6

Blood changes produced by the injection of hypertonic sodium chloride solution

RABBIT NUMBER	WEIGHT	TOTAL NUMBER OF DAYS WITHOUT FOOD OR WATER	RELATIVE HEMOGLOBIN VALUES		BLOOD TOTAL SOLIDS		BLOOD CHLORIDES				VOLUME OF 30 PER CENT NaCl INJECTED	DURATION OF INJECTION	TIME BEFORE DEATH
							Normal		At death				
			After fasting	At death	Normal	At death	Whole blood	Plasma	Whole blood	Plasma			
	K.				*grams/100 gms.*		*mgm. Cl/100 cc.*				*cc.*	*min.*	*min.*
016B											10	35	300
	2.4	0		99			296		474		5	30	0
St 2	2.5	9	136		18	16	286	355	541	650	10	15	10
St 4	2.0	9	156	108	18	16	289	379	591	731	15	55	20
St 6	2.1	9	110	98	18	16	284	356	679	795	10	40	10

4. Dehydration produced by hypertonic salt solution. In this aspect of the problem, four full-grown animals were employed. Three were deprived of food and water for nine days before intravenous injection of a 30 per cent sodium chloride solution; the fourth also received the intravenous injection but without previous deprivation of food and water. The results are detailed in table 6. The normal animal suffered a slight concentration of the blood from the first saline injection which was followed by dilution and return to normal within four hours; a further injection of 5 cc. of 30 per cent saline induced death in convulsions in a few minutes. Two of the fasting animals developed very high blood concentration as the result of fasting alone (values of 136 and 156) while the third developed a slight concentration (110). All these three animals died in convulsions soon (10 to 20 minutes) after the termination of the injection which lasted 20 to 40

minutes. Although the solution used was markedly hypertonic, the hemoglobin value at death gives evidence of a marked dilution of the blood.

TABLE 7

The average composition of the tissues in dehydration by hypertonic salt solution

TISSUES	ANIMALS	NUMBER	WATER CONTENT	ASH CONTENT	CHLORIDE CONTENT
			per cent wet tissue	*per cent wet tissue*	*mgm./100 grams wet tissue*
Lung	1*	9	80	1.22	168
	2*	3	75	1.55	371
	3*	1	79	1.24	206
Liver	1*	9	74	1.39	69
	2*	3	74	1.51	270
	3*	1	74	1.40	116
Skin	1*	9	75	0.75	172
	2*	3	65	0.80	260
	3*	1	64	0.71	216
Abdominal muscle	1*	9	78	1.24	53
	2*	3	74	1.22	161
	3*	1	74	1.28	
Leg muscle	1*	9	80	1.20	46
	2*	3	74	1.39	94
	3*	1	76	1.31	73
Kidney	1*	9	78	1.32	148
	2*	3	79	1.43	345
	3*	1	75	1.31	197
Small intestine	1*	9	81	1.35	113
	2*	3	81	1.41	277
	3*	1			
Spleen	1*	9	79	1.50	
	2*	3	76	1.89	
	3*	1	76	1.79	

1* Normal.
2* 9 days' fast—then saline injection.
3* Normal—then saline injection.

This dilution is real, for the blood solids in all three cases are markedly reduced, while the whole blood and serum chlorides are nearly doubled.

An examination of the tissues (table 7) of these animals discloses a decrease in water content of all the organs with the exception of the liver

and kidney. It is rather curious that the extent to which water was removed from the several tissues is generally of about the same magnitude in the normal animal and those starved nine days before the saline introduction. Yet the normal animal survived the first saline injection for a period of five hours, dying with the second injection, while the starved animals survived scarcely an hour from the time the injection was begun. Apparently the degree of dehydration produced in these animals is incompatible with life, but the rate at which the dehydration proceeds determines the length of survival. From a comparison of the relative decrease in the water content of the various organs, it is evident that the greatest relative loss of water occurs in the skin; the muscles and spleen lose relatively about the same amount. The large loss of water from the lung of the starved animals is surprising; it is the tissue richest in water in the body but it ordinarily plays only a minor rôle in water storage. A loss of five per cent of its normal water content must represent largely the essential water of the lung tissue. The increase of water in the kidney of the starved animals is well within the range of normal variation; still it contrasts with the unmistakable loss of water in the normal animal which survived the injection four hours longer. Apparently the early effort to flush out the salt by way of the kidney leads to such a depletion of the water stores that eventually the kidney is obliged to cease excreting and itself yield water to the concentrated blood.

The loss of water from the tissues is accompanied in nearly all cases by an increase in the ash content especially marked in the starved animals. This increase in ash content is not due to an increase in the chloride content; attention has already been called to the fact that the temperature of the electric muffle was high enough to volatilize completely all the chlorides. The greatest increase in the chlorides is in the liver where it is nearly four-fold; the lung, kidney, small intestine and abdominal muscle all approach a three-fold increase; the skin and leg muscle a two-fold increase. It is also a significant but obscure fact that the increase in chloride in the tissues was much greater in the fasted animals than the normal. The pronounced increase in the chloride content of the abdominal muscle over that in the leg muscle is at variance with the general belief that the metabolism of the musculature in all parts of the body is the same.

Conclusion. The slow intravenous injection of hypertonic saline solution produces a dilution of the blood in both normal animals and in animals deprived of food and water for several days, a marked loss of water from all the tissues except the liver (and possibly the kidney), and death within a short time.

5. Dehydration produced by hypertonic glucose solution. Five large rabbits were employed; one was normal; one was fasted without water for three days; two for eight days; and the other for nine days. The blood changes

show no definite tendency after the injection of 50 per cent glucose injection (table 8). Three of the five animals developed blood concentration, while the other two showed blood dilution as indicated by both the hemoglobin values and the total solids. Others (5) have found that glucose sometimes causes a blood concentration and sometimes a dilution. Lipschutz (6) reported low blood solids for a period of twenty-four hours after the injection of 60 cc. of 25 per cent glucose solution into normal animals; he observed that the blood chlorides diminished immediately after the injection but rose above normal in three hours and remained at a high level for some time. In the present experiment, the blood chlorides show no appreciable change. The normal animal survived the injection for eight hours and perhaps would have recovered although it evinced great weakness at the time it was killed. The fasted animals all developed respira-

TABLE 8

Blood changes produced by the injection of hypertonic glucose

RABBIT NUMBER	WEIGHT	TOTAL NUMBER OF DAYS WITHOUT FOOD OR WATER	RELATIVE HEMOGLOBIN VALUES		BLOOD TOTAL SOLIDS		BLOOD CHLORIDES				VOLUME OF 50 PER CENT GLUCOSE INJECTION	DURATION OF INJECTION	TIME BEFORE DEATH
							Normal		At death				
			After fasting	At death	Normal	At death	Whole blood	Plasma	Whole blood	Plasma			
	K.				*gms./100 gms.*		*mgm. Cl/100 cc.*				*cc.*	*min.*	*min.*
N11B	1.7	0		109	13.3	15.7	313		338	362	60	120	480
Sp D	2.1	3	100	94	20.3	19.2	259	351	235	265	42	120	40
Sp F	2.4	9	112	117	18.3	14.3			243	268	19	90	0
St 3	2.1	8	128	124	18.8	22.1	286	379	286	368	45	45	15
St 5	2.1	8	93		19.8	21.5	288	351	303	249	55	45	60

tory difficulty during the course of the injection (which lasted from 45 minutes to 2 hours), passed into convulsions, and died within an hour after the injection was stopped. These experiments were drastic and were planned with the intention of dehydrating the animals as quickly and as thoroughly as possible.

An inspection of table 9 reveals that the tissues of animals subjected to food and water deprivation lose considerably more water than those of the normal animal when treated with intravenous injection of hypertonic glucose solution. In the normal animal, the skin and leg muscles are the only tissues showing a significant change in water content, the skin standing preëminent. The fasted animals, on the contrary, have a low water content in all the tissues except the liver, kidney and small intestine. Their water content is also distinctly lower than the phlorhizinized animals fasted for the same length of time. Glucose in 50 per cent solution is much more

efficient as a dehydrating agent than 30 per cent saline, for the relative decreases are much greater with the former. The relative degree to which the tissues yield their water is the same except that the lung holds third

TABLE 9

The average composition of the tissues in dehydration by hypertonic glucose solution

TISSUES	ANIMALS	NUMBER	WATER CONTENT	ASH CONTENT	CHLORIDE CONTENT
			per cent wet tissue	*per cent wet tissue*	*mgm./100 grams wet tissue*
Lung	1*	9	80	1.22	168
	2*	1	80	1.37	168
	3*	4	75	1.28	150
Liver	1*	9	74	1.39	69
	2*	1	75	1.23	76
	3*	4	75	1.10	100
Skin	1*	9	75	0.75	172
	2*	1	67	0.77	216
	3*	4	60	0.55	133
Abdominal muscle	1*	9	78	1.24	53
	2*	1	78	1.32	66
	3*	4	70	1.12	68
Leg muscle	1*	9	80	1.20	46
	2*	1	77	1.45	50
	3*	4	72	1.31	62
Kidney	1*	9	78	1.32	148
	2*	1	78	1.38	160
	3*	4	78	1.14	131
Small intestine	1*	9	81	1.35	113
	2*	1			
	3*	4	81	1.21	139
Spleen	1*	9	79	1.50	
	2*	1	79	1.44	
	3*	4	75	1.54	

1* Normal.
2* Normal plus glucose solution.
3* Water and food deprivation plus glucose solution.

place instead of second. Hemorrhages of the lungs were found in only one of the animals receiving glucose, while all of the animals injected with saline had hemorrhagic lungs, and blood streamed from the nostrils of two

of them when they were stunned by a blow preparatory to killing. This may possibly explain the excessive dehydration of the lung produced by injection with saline. The ash content of the tissues of the glucose animals is generally decreased, that of the skin being greatest. There is no necessary relationship between the water lost and the ash content since in the skin the losses are in the same direction; in the lungs, however, the ash content is approximately equal to the normal although a considerable quantity of water has been lost. There is no decided change in the chlorides, except in the case of the skin where the chlorides are markedly decreased and in the liver where they are increased.

A rough calculation of the water lost from these animals, employing the figures of Skelton, gives a total loss of 128 grams of water, or 115 per cent of the total blood volume. The greatest portion of this comes from the skin and muscles.

Conclusion. Under the experimental conditions described, the injection of large amounts of hypertonic glucose solution may produce either a dilution or a concentration of the blood with little change in the blood chlorides.

The tissues lose much more water with glucose than with saline but in the same order, viz.: skin, lungs or muscle.

The water loss from the tissues in acute dehydration may assume figures greater than the total blood volume.

Discussion. These experiments corroborate the experience of others that the tissues tend to maintain constant their water content. That the tissues hold on their water doggedly beyond a certain limit can mean only one thing;—the reserve (free or interstitial) water has been exhausted and the tissues cannot release their essential water without serious damage to the function of the organs concerned. In the struggle for water between the blood and the tissues, the excretion of the urine and the secretions are checked and the metabolic rate is slowed down (Marriott).

It is not impossible, however, to force tissues to release their essential water, for, if a sufficiently strong stimulus is given, the whole nicely adjusted mechanism for water regulation can suffer a complete breakdown. Either blood concentration mounts until heart failure results, or else there is a sudden release of essential water from the tissues; but death occurs in either case. The drastic dehydration with saline and glucose represents an acute interference with all physiological processes leading to a disintegration of the tissues with an outpouring of the essential water of the tissues and immediate death.

The degree to which tissues give up water in anhydremic conditions bears no relation to the richness of water content. The lungs, intestines and spleen are rich in water, yet they lose little; while the skin, which is relatively poor in water, may lose one-fifth of its water. The osmotic relations prevailing in the different organs necessarily limit the quantity

of water that can be held free or bound. Any extensive release of bound water must involve a dysfunction owing to the changed properties observed when a hydrate or colloid loses the chemically combined water. There is abundant evidence that water never moves alone but always carries salts with it. After salt injections, the salt is removed with the water; when the blood volume is restored after hemorrhage, the chlorides are also restored but not quite so rapidly; the sweat and other secretions of the body, as well as exudates formed in response to injury are rich in salts, principally sodium chloride. In the present work with hypertonic saline, all the tissues gain in chloride and lose water. With hypertonic glucose, most of the tissues lose in water but the only marked alteration in chloride content is a decrease in the skin and an increase in the liver and muscle.

A study of the distribution of the chloride in the body reveals no definite relation between it and the water distribution. The blood and skin exceed all other tissues in chloride content yet they possess a relatively low water content. The muscles, which register so sensitively fluctuation in the degree of hydration of the whole organism and take so large a part in the water economy, are poorest in chloride of all the tissues. Pribram (7) has suggested that the mechanism which enables muscle to function normally despite fluctuations in the water content lies in the exchange of the potassium of the muscle for the sodium of the blood. The potassium salts give muscle its great absorptive power, while the exchange of potassium for sodium decreases the ability of the muscular protoplasm to hold water and so favor its release. This can be only a part of the story. As Adolph (8) has expressed it "no single substance will change the body's regulation of water content, though all of them are necessary for its maintenance. . . . The water is related to the whole of the body's constituents; it is part of the body's almost immutable structure."

There is no evidence that the tissues once parted from their essential water can continue to function even though water be supplied abundantly. This may be the reason why it is much easier to prevent blood concentration by fluid introduction than it is to decrease it once it has become established. Through dehydration not only are the properties of the protoplasmic colloids radically altered but their whole environment is changed through loss of salts, development of acidosis through anoxemia, etc. The constituents of the blood are altered at the same time. Under such circumstances the normal mechanism of fluid exchange between the blood and tissues cannot function. The reluctance of the tissues to yield water must mean one of two things or perhaps both:—either the permeability of the capillaries is altered so that fluid cannot pass outward from the tissues or else the release of water by the tissues would lead to a more profound interference with vital processes than a concentrated blood calls forth. There are no completely reversible reactions and few are even slightly so. Such

reactions in the animal organism as have lent themselves to study have followed the general physico-chemical laws operating in the inorganic world (9). But nowhere do such complicated and delicately balanced equilibria prevail as in the living body. If the addition of a little salt to a liter of water can change its boiling point, freezing point, electrical conductivity, solvent power, etc.; if a change in temperature determines the direction in which a reaction will go; if the drying out of a gelatin gel develops force enough to crack the glass plate on which it is dried, is it logical to assume that pathological tissues, in which all these changes have been shown to occur, will function like normal tissues? The answer to this question is given by the fact that under these conditions death occurs.

GENERAL CONCLUSION

Severe dehydration through water deprivation or by an increased osmotic power of the blood causes the tissues of the organism to lose their essential water with death as an invariable consequence.

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STUDIES ON THE MECHANISM OF WATER EXCHANGE IN THE ANIMAL ORGANISM

VIII. A Study of Dehydration by Pilocarpine under Varied Dietary Conditions

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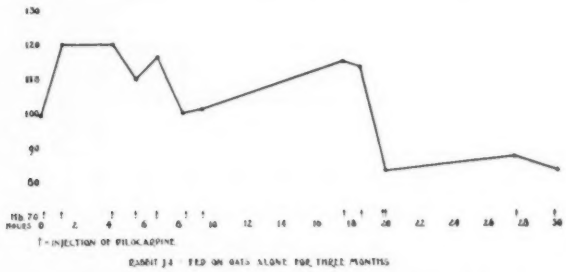
This work was undertaken as a result of a study made by Underhill and Freiheit (1) on the action of pilocarpine in the production of gastric ulcer, in which they found that the drug causes pulmonary edema and blood concentration. These facts led to the assumption that pilocarpine offers another method of producing dehydration through involvement of the nervous system.

The experiment was also planned to answer certain questions with respect to the influence of diet upon the capacity of the organism to hold reserve water and the release of the same under conditions of stress. Four groups of animals were subjected to the action of pilocarpine: *a*, 8 rabbits maintained on a stock diet (oats, cabbage, carrots, hay); *b*, 2 animals which had been fed corn meal and beets for a period of two months; *c*, 2 animals that had been maintained upon a diet of oats and carrots for two months; and *d*, 3 rabbits that had been fed oats alone for three months. The methods employed were those indicated in previous papers of this series.

Table 1 shows that the subcutaneous injection of large doses of pilocarpine (25 to 50 mgm. per kilo) produces an increase of blood concentration varying from 3 to 72 per cent. The concentration tends to return to normal values but can be maintained at a high level by giving pilocarpine every half hour. After the injections have been continued for several hours, the concentration begins to decrease (see graph). The fall coincides with the development of marked depression; the animal lies prone, jerking up its head spasmodically and grinding its teeth; respiration is barely perceptible; salivation generally ceases but sometimes continues. The degree to which the blood concentrates varies with the animal and can not

[1] A portion of these data is taken from the dissertation presented to Yale University by Merl E. Fisk in candidacy for the degree of Doctor of Philosophy, June 1929.

be increased by 6 to 8 days of fasting without water. The drug causes profuse salivation and the excretion of abundant unformed stools. Both responses generally diminish with the onset of depression and often stop altogether. Whether the influence of the diet is directly responsible or not, it was observed that the animals maintained for 2 to 3 months on a restricted diet (carrots and oats, cornmeal and beets, oats alone) show changes in response to the drug. Some are hypersensitive, a single injection causing convulsions followed by death within a few hours. Others resist very large doses of the drug, exhibiting only slight salivation and no purging. The loss of fluid through the secretions in these cases is small and a correspondingly mild degree of blood concentration develops. Although the hemoglobin values of these animals indicate that the blood has become diluted coincident with the onset of weakness, the total solids at death are greater than normal and the blood chlorides are usually somewhat in-



A TYPICAL HEMOGLOBIN CONCENTRATION CURVE
DURING PILOCARPINE TREATMENT

creased (table 1). Apparently the fluid that has diluted the blood is richer in solids than the normal plasma. The animals that die as the result of a single injection also show a precipitate fall of hemoglobin below the normal value.

An examination of the tissues (table 2) reveals that irrespective of the character of the diet, all animals show decreases in the water content of the skin, indicating that the fluid lost through the secretions comes from this source. The loss of water from the skin was most marked with animals maintained on a deficient diet (oats). The water loss on a diet of cornmeal and beets was less than when the animals were fed the stock diet. These animals had gained the most in weight. It would appear that if there is a definite relationship between fat and water storage, the fat animals are less capable of yielding their stored water or else there is less water available. The leanest animals,—those on the oat diet—lost relatively much more water. There is a distinct tendency for the organs,

TABLE I

The response of rabbits to pilocarpine injections under a variety of conditions

RABBIT NUMBER	BODY WEIGHT	GAIN OR LOSS OF WEIGHT	DIET	MONTHS ON DIET	DAYS WITHOUT FOOD AND WATER	DOSE OF DRUG	DURATION OF ACUTE EXPERIMENT	MAXIMUM HEMOGLOBIN RISE PER CENT OF NORMAL	BLOOD CHLORIDES				TOTAL BLOOD SOLIDS		REMARKS
									Normal		At death		Normal	At death	
									Whole blood	Plasma	Whole blood	Plasma			
	K.	*grams*			*cc. 5 per cent solution*		*hours*		*mgm. per 100 cc.*				*grams/100 cc.*		
FB	1.8		Stock		0	3	5	16							Abundant salivation. Loose stools
JA	1.9		Stock		0	4	4	72							Abundant salivation. Loose stools
FA	2.0		Stock		6	3		31							Fair salivation. Loose stools
FC	1.7		Stock		8	3	1.5	50							Fair salivation. Loose stools
JB	1.7		Stock		8	4	2	32							Fair salivation. Loose stools
JC	1.6		Stock		7	2.5	1.5	50							Abundant salivation. Loose stools
JD	2.0		Stock			4	3	54							Abundant salivation. Loose stools
F11	1.8		Stock		10	2.5	5	24	276	317	358	410	18.4	21.4	Slight response at first
SA	3.3	300	Cornmeal and beets	2		2	1		287		259				Salivation profuse. Little purging
SB	2.7	200	Cornmeal and beets	2		2	1	3	269		273				Salivation slight. Little purging
SE	1.7	−100	Oats and carrots	2		2	2.5	12	304		305				Salivation slight. Little purging
SF	1.4	−100	Oats and carrots	2		4	2.5	4	291		313				Salivation slight. Little purging

J1	1.1	−430	Oats	3		0.5	0.3	18	285	331	328	367	14.2	15.7	Instant death. Convulsion
J4	1.9	−350	Oats	3		6	31	21	294	334	365	412	18.0	21.5	Salivation slight. No purging
J2	2.6	40	Oats	3		8	33	18	290	343	312	372	17.3	19.4	Salivation slight. No purging

TABLE 2

Average composition of tissues in dehydration produced by pilocarpine

TISSUES	ANIMALS	NUMBER	WATER CONTENT	ASH CONTENT	CHLORIDE CONTENT
			per cent wet tissue	*per cent wet tissue*	*mgm./100 gms. wet tissue*
Lung	Normal	9	80	1.22	168
	Stock diet	8	80	1.19	
	Cornmeal and beets	2	83	1.20	151
	Oats and carrots	2	80	1.28	
	Oats	3	79	1.32	132
Liver	Normal	9	74	1.39	69
	Stock diet	8	75	1.14	89
	Cornmeal and beets	2	82	1.19	104
	Oats and carrots	2	78	1.36	110
	Oats	3	73	1.35	101
Skin	Normal	9	75	0.75	172
	Stock diet	8	66	0.71	176
	Cornmeal and beets	2	71	0.58	180
	Oats and carrots	2	64	0.57	227
	Oats	3	62	0.59	159
Abdominal muscle	Normal	9	78	1.24	53
	Stock diet	8	75	1.18	93
	Cornmeal and beets	2	77	1.18	49
	Oats and carrots	2	82	1.17	81
	Oats	3	69	1.07	68
Leg muscle	Normal	9	80	1.20	46
	Stock diet	8	77	1.20	48
	Cornmeal and beets	2	79	1.22	43
	Oats and carrots	2	80	1.10	67
	Oats	3	75	1.16	54
Kidney	Normal	9	78	1.32	148
	Stock diet	8	77	1.31	127
	Cornmeal and beets	2	81	1.14	125
	Oats and carrots	2	81	1.21	161
	Oats	3	80	1.11	166
Small intestine	Normal	9	81	1.35	113
	Stock diet	8	82	1.14	241
	Cornmeal and beets	2			
	Oats and carrots	2			
	Oats	3	76	1.40	167
Spleen	Normal	9	79	1.50	
	Stock diet	8	78	1.47	
	Cornmeal and beets	2	80	1.76	
	Oats and carrots	2	82	1.46	
	Oats	3	75	1.66	

except the skin, to show an increased water content when the animals are maintained on a diet of fresh vegetables (beets or carrots). On the other hand, the tissues of animals fed oats alone lose water in every case except the kidney. Both groups developed slight salivation and purging in response to the pilocarpine, but, whereas the animals fed green vegetables collapsed within a few hours, those on the oat diet withstood the treatment for more than 30 hours. It is quite possible that the fluid losses over such an extended period were sufficient to cause the observed decrease in tissue water content even though the degree of response to the drug was slight. The increased water content in the other animals resembles the experience gained that avitaminosis is accompanied by edema of the tissues (2). Krause (2) noted an increased water content of the tissues of pigeons and guinea pigs maintained on a diet low in vitamin B, and Terroine, Fuerbach and Brenckmann (3) found that the increase in water content of mice suffering from avitaminosis resembles that of starvation. In the present experiments, the increased water content of the tissues may be the result of autolytic processes to supply lacking dietary elements. Both fat and lean animals exhibit it. The animals kept on oats and carrots lost weight and were obviously thin, while those on cornmeal and beets increased in weight and had an abundance of fat throughout the body. It is the general experience that fat animals contain less water than lean animals, owing to the fact that fat can be stored with less water than any other reserve substance (4). The fat animals in this series all exhibit an increased water content especially in the liver, kidney and muscles. This increase in part may be due to the fact that the results are not reported on a fat-free basis, but it is not due wholly to this because the lean animals show the same tendency to a smaller degree.

A difference in the response of the abdominal and leg muscles is evident wherever the water reserve of the muscles has been utilized to any extent, for, in each instance, the abdominal muscle yields considerably more water than does the leg muscle. The alterations in the chloride and ash contents of the various tissues exhibit no definite trends; fluctuations are within the normal limits for the most part, although the chlorides of the liver and of the skin tend to be high.

SUMMARY

The repeated injection of pilocarpine causes the maintenance of a highly concentrated blood which, however, returns to normal as soon as the administration of the drug is stopped.

After prolonged treatment with pilocarpine, the concentration of the blood suddenly decreases to a level below normal, coincident with the onset of collapse.

Animals in poor nutritive condition are either hypersensitive to the drug or extremely refractory.

The most significant changes in the water content of the tissues under the influence of pilocarpine are: *a*, a decrease in the skin, especially marked in lean animals, indicating that the water reservoirs of the skin yield the fluid for the secretions induced; *b*, an increase in most of the organs of animals receiving fresh vegetables as part of the diet.

The alterations in ash and chlorides content show no constant trend.

It is indicated that the character of the diet has a direct influence on the ability of the tissues to yield water. In general, a lean animal yields more water from its tissues than does a fat one.

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CIRCULATORY CHANGES DURING PERIODIC BREATHING PRODUCED BY MODERATE OBSTRUCTION TO THE CEREBRAL ARTERIES[1,2]

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The study of periodic ventilation has been approached from many viewpoints. Perhaps the first clear reference to the condition as observed in man was that given by Hippocrates (Adams, 1849) when he described, what we now know to be periodic breathing, in the following words, "The respiration throughout like that of a person recollecting himself, was rare and large." Since the time of Hippocrates a large number of papers have appeared dealing with this phase of ventilation. It is not within the scope of the present paper to review the numerous theories and experimental data which have a bearing on this peculiar respiratory phenomenon. However, we would like to call particular attention to some of those works which have a more or less direct bearing on the present study. One of the outstanding of these is that of Eyster (1906), who by means of increased intracranial pressure experimentally induced excellent examples of periodic breathing. He pointed out that, "A sudden increased supply of blood to a respiratory center which has its irritability considerably depressed as the result of long continued insufficient blood supply, but is nevertheless still capable of response, may result in apnea;" and that "Cheyne-Stokes respiration in states of increased intracranial tension, with blood pressure waves rising and falling above and below the line of intracranial tension, is due to periodic activity of the respiratory, vasomotor, and cardio-inhibitory centers, the underlying cause of which is an alternate anemia and blood supply to the medullary centers."

Douglas and Haldane (1909) after experiments on man and animals with overventilation or breathing through a long tube with or without a soda lime cartridge, came to the conclusion that, "Periodic breathing is produced by the periodic occurrence and disappearance of the excitatory effects of want of oxygen on the respiratory center. The want of oxygen may be due to abnormal deficiency in the alveolar oxygen pressure or it

[1] Preliminary report appeared in the Proceedings of the Society for Experimental Biology and Medicine, 1929, xxvi.

[2] A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

may be due to effects in the circulation, or changes in the breathing or to both causes combined."

Macleod (1921) induced periodic breathing in decerebrate cats by a number of procedures which included the periodic compression of the vertebral arteries. He pointed out that, "The arterial blood pressure exhibited waves synchronous with those of the respirations, and occasionally there is evidence of vagus slowing of the heart during the rising part of the curves. The arterial waves are often relatively much more pronounced than the respiratory and occasionally the former may be marked without any detectable periodicity of breathing."

The emphasis placed by Gesell (1923) on the significance of the volume flow of blood in relation to the metabolism, the state of oxidation, and the acid base equilibrium of the respiratory center as effected by the transport of oxygen and carbon dioxide of the blood, suggested a more detailed experimental analysis of periodic respiration.

As far as we have been able to ascertain, the exact nature of blood volume flow changes during periodic breathing has not been determined. In order to gain a clearer conception of this relationship, blood volume flow was measured not only to the brain but to other parts of the body as well, to correlate peripheral and central changes.

METHOD. Dogs anesthetized with morphine and urethane were used for all experiments. Unless otherwise stated, 0.5 cc. of a 2 per cent solution of morphine and 1 gram of urethane per kilogram of body weight was used. Periodic breathing was induced in all cases by limiting the cerebral flow of blood. This was accomplished by the gradual occlusion of the carotid and vertebral arteries. The blood of one of these arteries, before reachng the head, passed through a compression chamber which is illustrated in figure 1. This consists of an inner rubber tube, *C*, surrounded by a *T* shaped glass tube. The rubber tube was prepared by cementing together the edges of two strips of rubber dam. The circulating blood passed through this tube. A mercury manometer arranged to record the pressure in the glass chamber is in series with *A* and a Mariotte bottle. When pressure is applied, the outflow *B* is closed. Roughly speaking, if the pressure in the chamber surrounding tube *C* is higher than the mean blood pressure, the walls of tube *C* are pressed tightly together without wrinkling and allow no blood to pass. It follows, therefore, that during rhythmical waves in blood pressure which rise above and fall below the occlusion pressure, periodic variations in blood flow to the head occur. The compression chamber, therefore, supplies us with a method for readily controlling blood flow to the head without the introduction of a foreign fluid into the subdural spaces or increasing the volume of blood from the absorption of the compression fluid which is used to increase the intracranial pressure. These changes in blood flow are similar in many respects to those obtained

by the intracranial pressure method employed by Eyster (1906). When arterial compression is used, only one arterial flow is automatically controlled. The remaining arteries are occluded or deoccluded as the conditions demand.

The thermoelectric method of Gesell and Bronk (1926) was employed for measuring the blood volume flow changes in the arterial streams. It was found desirable, on account of the importance of time relationships, to resort to a very small thermopile vessel. This reduces the lag between blood flow changes and thermopile response. Figure 2 is a sectional view of the thermopile vessel which was interposed between the heart and compression chamber. It differs from a modification described by Gesell (1929), in the absence of an outer vacuum jacket and its marked reduction in size. The thermopile was attached at *A* and *B*, one side being employed for the cold junctions and the other for the hot. The inner tube *C* is a very thin glass tube through which the arterial stream flows. The distance between *A* and *B* is 25 mm. and the outer jacket for water which surrounds

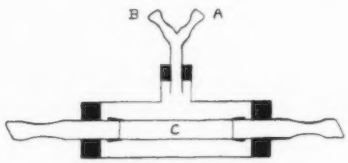


Fig. 1

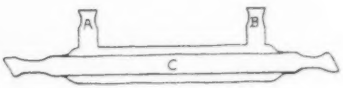


Fig. 2

tube *C* is only 7 mm. in diameter. Allowing water from a Mariotte bottle to flow through at the rate of 20 cc. per minute, the lag of the instrument was found to be about 4 seconds. On all the records an upstroke indicates an increased blood flow and a down stroke a decreased flow. Any line marked *X* signifies mechanical adjustment of the writing point to keep it on the record. A type K Leeds and Northrup potentiometer was employed and connected in the usual manner by thread and pulley to the writing point. In figures 3A, 3B, and 3C, the coarse adjustment of the potentiometer was used, but for figures 4A, 4B, 4C, 4D, and 4E, the fine setting was used. The fine setting was especially desirable for recording the small variations in flow of the femoral artery, as it increased the sensitivity ten times.

The venous returns of the external jugular, vertebral, and femoral veins was measured by the enclosed drop method of Gesell (1929). This method has the distinct advantage of allowing automatic return of the blood to the vein and at the same time keeps the blood volume constant. It is desirable to have as many drops as possible per minute in order to record the smallest variations in volume flow. Therefore, the smallest sized drop compatible

with the extent of the volume flow was employed in each vein. For example, in the femoral vein, a very small drop was used since the flow was not rapid, but a much larger drop was required in the external jugular vein where a large volume of blood was flowing. To prevent clotting, 40 mgm. of heparin per kilogram of body weight were used. This was injected at the beginning of the experiment, the heparin being dissolved in about 50 cc. of warm isotonic saline. When the enclosed drop method was used, partial defibrination of the blood before the addition of the heparin tended to prevent streaming of the blood through the oil. This partial defibrination was accomplished by means of small hemorrhages and reinjection. In the case of a very small intestinal vein, the external drop method was used and recording accomplished through a system of tambours.

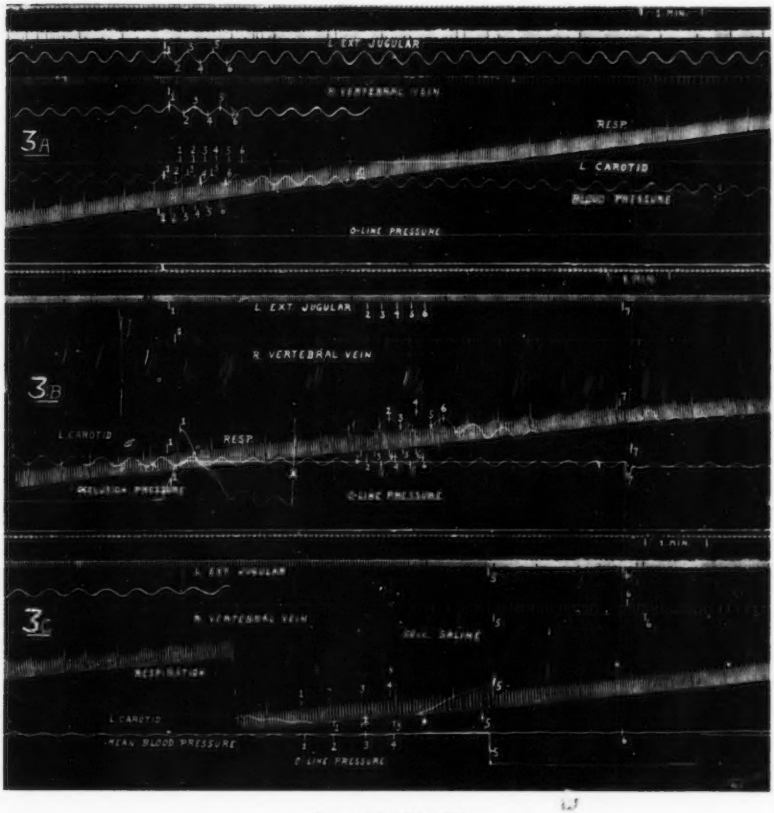
Blood pressure, ventilation, oxygen consumption, and time were simultaneously recorded with the blood volume flow changes. Corresponding points on these different measurements are designated by the corresponding numbers on the figures.

Results. A study of the literature reveals a surprising number of experimental procedures which is perhaps indicative of the difficulty in producing periodic ventilation. We finally adopted simple occlusion of arteries supplying the head in conjunction with the occlusion chamber described above, as the most desirable method for the present study. Some dogs apparently had a marked susceptibility to periodicity. If respiration failed in these animals they could usually be revived by artificial ventilation and deocclusion of the arteries supplying the head. Upon curtailing the blood flow to the head, periodic ventilation frequently returned.

As a result of our experiments, we have found periodic ventilation to fall into two distinct types. One type is characterized by rhythmical changes in depth and rate of respiration without apneic periods. This type is considered in this paper. The other type which will be fully considered in the second paper, has distinct apneic periods varying from a few seconds to two minutes or more in duration and is produced by greater impairment of circulation.

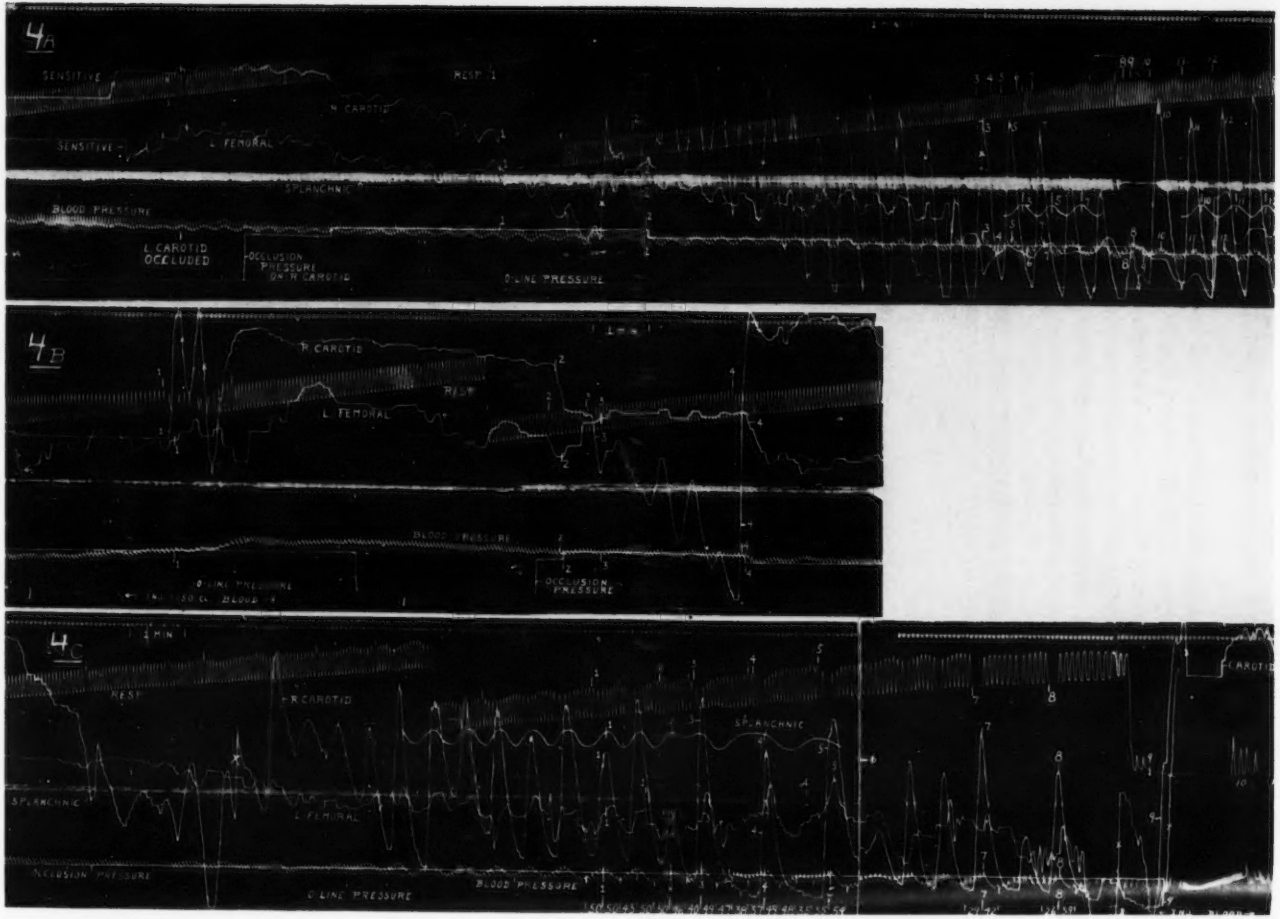
Figures 3A, 3B, and 3C represent a series of records in which several blood flows were measured. The vertebral arteries, the left vertebral vein, the right external jugular vein, were occluded before any records were taken. The right common carrotid artery was patent during the entire experiment. The blood in the left common carotid artery flowed through the thermopile vessel and the compression chamber before reaching the head. The venous returns of the left external jugular and right vertebral veins were followed by the enclosed drop method. The flow of blood of the right vertebral vein was returned to the heart through the right external jugular vein which was peripherally occluded.

We do not know when periodicity first occurred in this experiment as it was present at the beginning of the first record (fig. 3A) and occurred without application of pressure to the left carotid artery. In figure 3A, below the records of the flows of blood of the left external jugular vein and right vertebral vein, periodic variations in flow were diagrammatically plotted. A careful inspection of the thermoelectric record of the left common

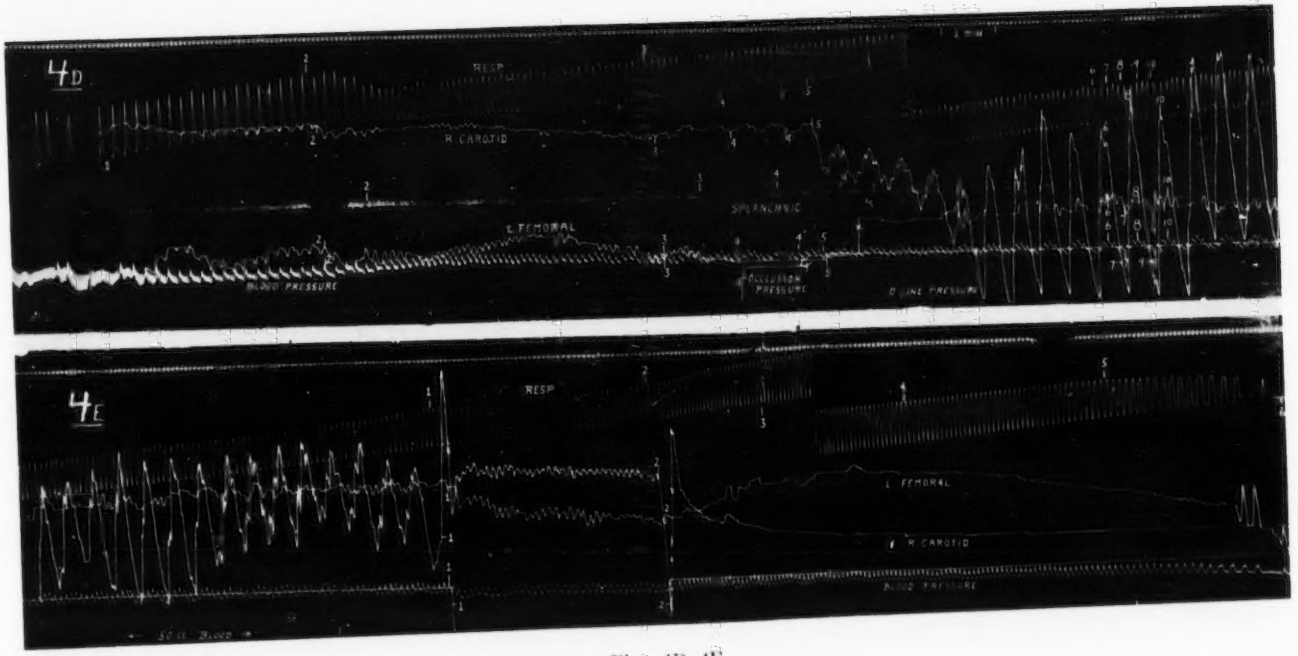


Figs. 3A, 3B, 3C

carotid arterial flow revealed only slight rhythmical variations. These waves although very small represent significant variations in flow as the coarse adjustment of the potentiometer was used. As indicated by the corresponding numbers, 1, 3, and 5, the highest venous and arterial flows all occurred at the crests of the mean blood pressure waves. At the troughs of the mean blood pressure waves 2, 4, and 6, the lowest arterial and venous flows occurred. Respiration had a very smooth even periodicity through-


4A
SENSITIVE
RESP.
R. CAROTID
SENSITIVE
L. FEMORAL
SPLANCHNIC
BLOOD PRESSURE
L. CAROTID OCCLUDED
OCCLUSION PRESSURE ON R. CAROTID
0 LINE PRESSURE
4B
R. CAROTID
RESP.
L. FEMORAL
BLOOD PRESSURE
0 LINE PRESSURE
OCCLUSION PRESSURE
4C
1 MIN.
RESP.
R. CAROTID
SPLANCHNIC
SPLANCHNIC
L. FEMORAL
OCCLUSION PRESSURE
0 LINE PRESSURE
BLOOD PRESSURE

Figs. 4A, 4B, 4C



Figs. 4D, 4E

out this figure, with the lowest depth and slowest rate occurring a few seconds after the highest blood pressures and blood flows. This relationship appeared through this experiment. The vertebral venous flow soon became too slow to show changes in periodicity accurately. The gradual fall in blood pressure was accompanied by a slowing of the venous returns of the left external jugular and right vertebral veins as well. Periodicity of the recorded vertebral flow was plotted only up to the middle of figure 3A.

At number *1* in figure 3B, the left carotid was partially occluded by increased pressure in the compression chamber. The thermoelectric record of the arterial flow showed a decreased volume flow. This same decrease in flow occurred in the right vertebral and left external jugular veins. The depth of respiration gradually increased following compression number *1*. This was followed by an increased periodicity of ventilation during which the same relationship existed between blood pressure and blood flow and respiration as pointed out in figure 3A. Thus respiration shows the lowest depth and rate following the highest arterial pressures and flows *2*, *4*,and *6*, and the highest depth and rate at the lowest arterial pressures and flows *3* and *5*. Oxygen consumption was only slightly less in this figure than in figure 3A. This was shown by the smaller rise in the base line of respiration. Total ventilation was increased approximately 25 per cent by the application of the occlusion pressure. In other words, the general metabolism of the body had not changed materially but the respiratory center was manifesting a local stimulation associated with diminished blood pressure and blood flow.

At *7* the occlusion pressure was slightly lowered as the crests of the blood pressure waves were failing to rise appreciably above the line of occlusion pressure. A significant increased carotid flow took place which was associated with a temporary decrease in respiration.

During the first half of figure 3C, blood pressure waves were slight as compared with those in figure 3A. The carotid flow, likewise manifested a decrease in amplitude of the waves and the level of flow slowly fell. Periodicity in respiration was very much diminished at *1* and *2*. The slow injection of saline at *3* brought about a definite decrease in ventilation as soon as the mean blood pressure rose above the occlusion pressure. Removal of occlusion at *5*, increasing the volume flow of blood still more, was followed first by a short apnea which then gave way to increased ventilation indicative of a general recovery of the respiratory center.

Figures 4A, 4B, 4C, 4D, and 4E show the results of another experiment. Both vertebral arteries were permanently occluded. Thermopile vessels were interposed in the blood flows of the right common carotid and left femoral arteries. Variations in flow were followed in each by the sensitive setting of the potentiometer. One of the smaller intestinal veins was can-

nulated for recording the flow of blood by the external drop method. The blood was not defibrinated, only heparin being used to prevent clotting.

This animal developed periodic breathing to a marked degree. The beginning of the record is shown in figure 4A. Occlusion of the left common carotid had no influence on respiration but waves in blood pressure developed. The slow continuous hemorrhage entailed by recording the splanchnic flow of blood in itself decreased mean blood pressure and cerebral blood flow and may be partly responsible for the development of periodicity. Further occlusion of the right carotid brought on larger oscillations in blood pressure and volume flow of blood but did not bring on an immediate change in respiration. As the mean blood pressure fell below the occlusion pressure at *1*, the flow in the right carotid sharply decreased. The femoral flow, however, exhibited only a gradual decline. At *2* a slight lowering of the occlusion pressure brought about a marked increase in carotid flow which was associated with a slight decrease in respiration. Femoral flow decreased slightly. This was probably due to the fall in mean blood pressure. With the establishment of this new level in occlusion pressure, waves in blood pressure became very marked. The variations in carotid flow were of great magnitude. The irregular respiration became distinctly periodic at *3*. The relation of respiration to blood flow and mean blood pressure is shown by the corresponding numbers *3*, *4*, *5*, *6*, and *7*. Both femoral and splanchnic flows were greatest at the crests of the blood pressure waves and ventilation was the smallest. A small increase in carotid flow at *8* was associated with only a slight decrease in the rate and depth of respiration. At *9*, a lowering of the occlusion pressure was accompanied by a larger increase in flow of blood and a greater depression of ventilation. Following this the oscillations in volume flow became larger despite the fact that the oscillations in mean blood pressure were smaller. These changes were associated with a more marked periodicity in ventilation.

During the time required to change smoked papers, mean blood pressure had fallen below the line of occlusion pressure and prominent waves in blood pressure and respiration had disappeared. This condition is seen at the begininng of figure 4B. A slow injection of the blood was begun at the extreme left of the figure and just prior to *1*, ventilation gradually diminished along with the increased blood pressure and blood flow to the medullary centers presumably by way of the spinal anastomoses. At *1*, when mean blood pressure was high enough to overcome the pressure in the occlusion chamber, a large carotid blood flow occurred. Immediately following this, there was a marked decrease in ventilation which then gave way to an even ventilation. At *2*, the occlusion pressure was raised again to equal the mean blood pressure. Femoral flow increased considerably although there was only the slightest increase in mean blood pressure.

Coincident with this there was a marked reduction in carotid flow, but no appreciable change in ventilation. From *3* to *4* there were slight waves in mean blood pressure which were associated with moderate variations in carotid flow. Although slightly more irregular, respiration did not show distinct periodicity. Partial de-occlusion of the carotid at *4* was accompanied by increased carotid flow of blood, decreased mean blood pressure and decreased ventilation. With a gradual deterioration of the animal there was a gradual fall in mean blood pressure which automatically increased the occlusion of carotid artery in record 4C with the new occlusion pressure established at *4* in record 4B. When the occlusion pressure and mean blood pressure began to coincide periodicity was established and became more pronounced as the mean blood pressure fell and as the animal continued to deteriorate. Toward the close of the record pulse rate became periodic and apneas appeared. The results began to resemble more closely those produced by marked impairment of circulation described in the following paper.

Between figures 4C and 4D, seven minutes elapsed during which the respiration was irregular. At number *1* of figure 4D this irregularity suddenly changed to very deep regular breathing. At *2* the left carotid was de-occluded and within the next minute respiratory rate increased and depth decreased. Re-occlusion was begun at *4*. There were no immediate effects until the occlusion pressure was equal to the mean arterial pressure, *5*. Then periodicity in mean blood pressure and carotid flow was established and as the periodic changes in mean blood pressure and carotid flow of blood increased in magnitude they were associated with greater changes in ventilation. De-occlusion at *1* in record 4E results in a general decrease in ventilation and re-occlusion at *2* in a general increase. There is then a gradual impairment of respiratory function which presumably may be attributed to the reduction in cerebral flow of blood, attempts at resuscitation by injection of blood failed.

Discussion. Granting that the activity of the respiratory center is sensitive to changes in volume flow of blood by virtue of chemical changes occurring in the center the periodic changes in ventilation have a simple explanation in the changes in cerebral flow which are here definitely demonstrated to exist. It is only essential to apply the principles of chemical stimulation of the respiratory center to the circulatory centers (Gesell, 1929) to complete the explanation. But the recent findings of Heymans and Bouckaert (1930) demonstrating depression of respiratory movements with increased pressure in the carotid sinus suggest that reflexes initiated by alternate stretching and contraction of the carotid sinuses and arch of the aorta might well play a part in the periodicity of ventilation, perhaps even be self sustaining in maintaining periodicity in circulation and respiration. The relative importance of central chemical and peripheral vascular reflex effects during periodicity requires further study.

SUMMARY

Periodic breathing was induced in dogs under morphine and urethane anesthesia by moderate occlusion of the cerebral blood vessels. This was accomplished by placing one of the common carotid arteries in a compression chamber, thus making possible partial or complete occlusion of this artery. The remaining cerebral arteries were occluded and de-occluded as conditions required.

Pulmonary ventilation and mean blood pressure were recorded. Volume flow of blood was followed in the carotid and femoral arteries thermoelectrically with small thermopile vessels of short lag. The vertebral and external jugular venous flows were recorded by the enclosed drop method and intestinal venous flow by the external drop method.

Periodic changes occurred in mean blood pressure, in femoral and carotid arterial blood flows, in vertebral, external jugular, and splanchnic venous flows, and in depth and rate of respiration. Changes in blood flows were synchronous with the periodic variations in mean blood pressure. In contrast to this general periodicity there were no obvious rhythmical changes in heart rate. Ventilation increased during decreasing blood pressures and blood flows, and decreased with increasing pressures and flows. With the conditions provided no apneas developed.

The absence of periodic changes in heart rate suggested that the changes in mean blood pressure may be due to periodic activity of the vasomotor center.

It is concluded that the periodic changes in ventilation are related to the periodic changes in the circulatory system but since volume flow of blood and mean blood pressure vary with each other the periodic changes in ventilation may be a result of periodic chemical changes in the respiratory center produced by fluctuations in blood flow or to the reflex effects of periodic stretching and contraction of the aorta and of the carotid sinus. In the absence of crucial experiments the relative importance of these two effects cannot be evaluated.

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CIRCULATORY CHANGES DURING PERIODIC VENTILATION WITH APNEAS PRODUCED BY MARKED CURTAILMENT OF BLOOD FLOW TO THE BRAIN[1,2]

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In the previous paper a form of periodic respiration was described, characterized by periodic changes in ventilation with apneas virtually lacking. The present paper deals with periodic ventilation with prolonged apneas, produced by a greater impairment of circulation than employed in the previous paper.

METHODS. The experiments were carried out on dogs which were anesthetized with morphine and urethane or with amytal. Periodic ventilation was brought about by one of the three methods: 1, ligation of spinal arteries in conjunction with method 3, virtually giving complete control of the blood flow to the head. This method was used in obtaining figures 2A and 2B. It was adopted because the spinal artery is often alone sufficient to supply an adequate amount of blood to maintain even respiratory function; 2, increased intracranial pressure, by the use of a brass tube inserted into a trephined opening in the parietal bone with the dura mater incised to permit passage of fluid under pressure into the subdural spaces. This procedure is essentially the same as that reported by Eyster (1906). This method was used in obtaining figure 3; 3, occlusion pressure applied to a cerebral artery by the use of a pressure cuff surrounding the vessel (this was considered in detail in the previous paper) along with occlusion of other arteries. This method was used in obtaining figures 4A and 4B.

The operative procedure in the first method is briefly as follows. The vagus nerves and the common carotid arteries were isolated and kept intact. In the region of the second cervical vertebra, the vertebral arteries were ligated and divided at their exit from the vertebral canal and all remaining tissue exterior to the second cervical vertebra excepting the common carotids and vagus nerves was divided by cautery. The posterior bony arches of the second cervical vertebra were then cut by means of

[1] Preliminary report appeared in the Proceedings of the Society for Experimental Biology and Medicine, 1929, xxvi.

[2] A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

an electric rotary saw, the spinous process removed, and the dura mater incised. By placing a silk ligature around the dentate ligaments, the cord was elevated sufficiently to secure a ligature around the spinal artery. The smaller spinal arteries lying in the lateral sulci were then ligated. The venous return from the head was maintained by the spinal sinuses. To prove the effectiveness of this method in controlling the blood flow to the brain, the operation just described was performed and all cerebral arteries in the region of the second cervical vertebra were occluded and heparin added to prevent coagulation of the blood. Within a few minutes after the death of the animal, an aqueous solution of eosin was injected under 120 mm. mercury pressure into the trunk of the animal by way of the femoral arteries. The entire trunk of the animal became stained as well as the cord posterior to the level of the tie of the spinal arteries. The cord anterior to the tie remained unstained. This staining is shown by the black shading in figure 1 in the longitudinal and cross sectional views of the cord at different levels.

The methods described in the previous paper for recording blood pressure, respiration, the electrometric recording of blood flows, etc., are the same as those used in the present paper.

Results. Figures 2A and 2B are continuous records of one experiment. The experimental procedure was method 1 which included the ligation of the spinal arteries. Records were obtained of femoral arterial mean blood pressure, respiration, occlusion pressure applied to one carotid artery, left common carotid blood volume flow, femoral arterial blood flow, and time in seconds. The continuous thermoelectric method was utilized for the blood volume flows. The anesthetic used was urethane and morphine.

Previous to figure 2A, the right carotid artery was occluded leaving the left carotid artery as the sole source of blood to the head. Figure 2A shows the application of occlusion pressure on the left carotid at *1*, *2*, *3*, and *4*. The occlusion pressure at *1* was raised to a point considerably below mean blood pressure. This occlusion pressure is maintained until point *2*. During the period *1* to *2*, there is no significant change in the carotid or femoral blood flows, heart rate, mean blood pressure or respiration. At *2* the occlusion pressure is raised slightly higher and maintained at this new level until point *3*. Respiratory minute volume is increased thirty per cent coincident with the significantly lowered carotid blood flow. Heart rate, mean blood pressure, and femoral blood flow are not altered appreciably. A further decrease in carotid blood flow is obtained by again raising the occlusion pressure at *3*. This new level of occlusion pressure was maintained through the period *3* to *4* and was accompanied by a decrease in carotid flow, no changes in mean blood pressure and a further increase of respiratory minute volume of fourteen per cent, or an increase over the preocclusion pressure period (to the left of *1*) of 44 per cent. Femoral blood

flow and heart rate are not significantly altered during this period. At *4* the occlusion pressure was raised to equal mean blood pressure. Following this procedure the mean blood pressure rose slightly, but fell below the occlusion pressure at *5*. During the early part of the period *4* to *5* respiratory stimulation increased still further, but the remainder of this period showed respiratory depression. At the onset of period *5* to *6* mean blood pressure fell below the occlusion pressure. This period was accompanied by marked decreased carotid flow and decreased heart rate. In the early part of this period, *5* to *6*, respiration terminated in apnea, lasting

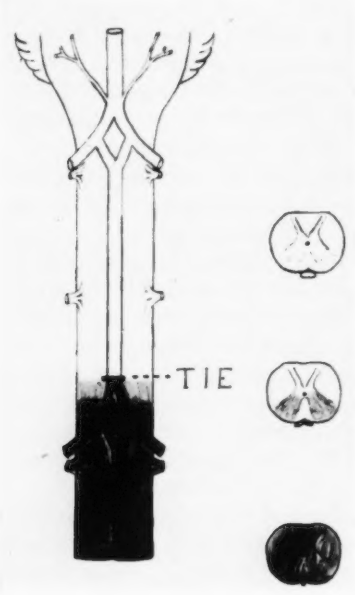
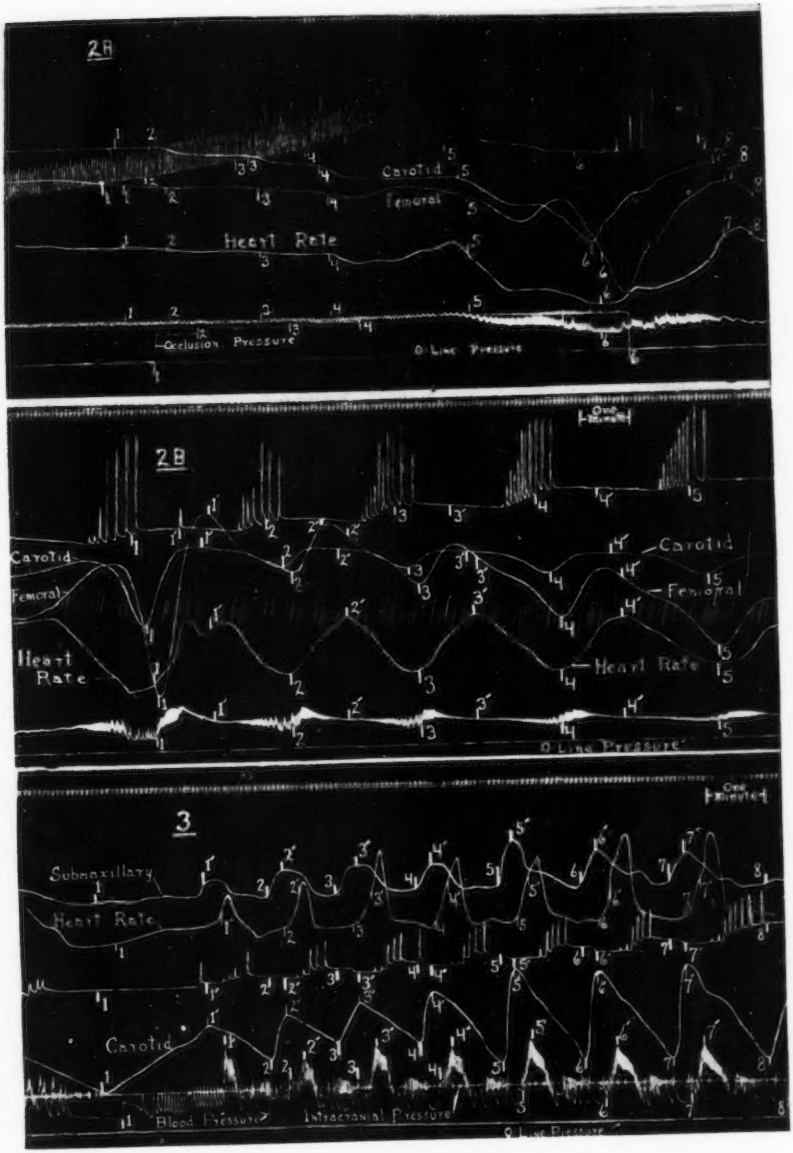


Fig. 1

two minutes and seventeen seconds. The occlusion pressure was removed at *6*, and twenty seconds later a group of respiratory movements was initiated. This group of thirteen respirations began just before the deocclusion of the right carotid at *7* and the injection of 100 cc. of glucose-saline at *8* with a small depth and increased to a maximum. Ventilation then decreased ending in apnea. Apnea, slow pulse rate, reduction in blood flow and mean blood pressure go hand in hand. With removal of occlusion pressure at *6*, increase of heart rate, blood flow and ventilation follow coincidently.


2A
Carotid
Femoral
Heart Rate
Occlusion Pressure
0 Line Pressure
2B
Carotid
Femoral
Heart Rate
0 Line Pressure
3
Submaxillary
Heart Rate
Carotid
Blood Pressure
Intracranial Pressure
0 Line Pressure

Figs. 2A, 2B, 3

In figure 2B, periodic ventilation is well established. Corresponding points marked on all of the records show that pulmonary ventilation of increasing volume occurs during a period of decreasing mean blood pressure and decreasing carotid and femoral flow of blood. Toward the close of these periods of increasing ventilation, mean blood pressure and volume flow of blood increase and after 1, 2, or 3 breaths apnea is suddenly established. The final breaths show a distinct decrease in rate. Periodic changes in heart rate (minimum 60, maximum 204) are visible on the blood pressure tracing and are plotted in the overlying curve. Heart rate corresponds more accurately with volume flow changes in the carotid artery than with changes in mean blood pressure, possibly indicating a preponderance of central chemical control over arterial reflex control. It will be seen that increased heart rate comes on during increased cerebral flow of blood and after increased pulmonary ventilation. It might, therefore, be interpreted as a result of recovery of the cardio-inhibitory center from previous stimulation produced by a combination of apnea and decreased flow of blood.

The sequence of events which present themselves in figure 2B, may possibly be interpreted as follows. Respiratory stimulation occurs during and as a result of the decreased brain blood flows which are accompanied by decreasing heart rate from increased vagal tonus. The oxygen supplied by the increased ventilation removes the central vagal inhibition and the heart rate increases. This is accompanied by an increased blood flow to the brain which should lead to a further recovery of the inhibitory centers and a still greater increase in mean blood pressure and volume flow of blood. The excessive chemical changes in the respiratory center from increased flow of blood and accentuated ventilation results in apnea. The next blood to leave the lungs going to the brain is now deficient in oxygen and contains more carbon dioxide than it did during the respiratory period. Stimulation of the inhibitory center effects a decrease in heart rate and a decrease in blood flow to the brain as the blood pressure falls. The stimulation of respiration is finally brought about by the cumulative effects of lack of oxygen and the increased acidity resulting from apnea which is accentuated by the significant decrease of blood flow to the brain.

Figure 3 is an example of periodicity produced by increased intracranial pressure. Femoral blood pressure, respiration, the venous return from the submaxillary gland followed by the external drop method, the left common carotid blood flow followed by the thermoelectric method, intracranial pressure, and time in seconds were simultaneously recorded.

Previous to figure 3, intracranial pressure was raised to a point slightly below that of mean blood pressure. There was temporary stimulation of respiratory movements which gave way to gradual depression terminating in apnea of two minutes and forty-seven seconds' duration. The last three

respirations preceding this initial apnea are shown in the figure. Following the single respiratory movement at the end of this apnea, periodicity is thoroughly established. This initial respiratory movement occurs during the increased flow of blood and increased mean blood pressure, and in this respect resembles the group of increasing respiratory movements to the right of *6* in figure 2A, which also occurs during recovery with increasing blood flow. Following this initial respiratory movement there is a sudden increase in mean blood pressure which undoubtedly is accompanied by a greater increase in blood flow than that indicated on the volume flow record, for the thermopile vessel used in this experiment had considerable lag. Coincident with this suddenly increased blood pressure apnea occurs, and this may possibly be compared again with the development of apnea in figure 2A which followed augmented blood flow upon deocclusion of the carotid artery. Following the sudden increase in blood pressure, the first periodic fall in blood pressure occurred, and this is accompanied by the first respiratory group of periodicity. It, therefore, appears that the initial respiratory movement *1′* is interposed between a depression and a recovery apnea. (These terms will be amplified.) If true, it indicates how quickly depression, recovery, and stimulation give way to each other in the respiratory center.

In analyzing the established periodicity, the maximum and minimum carotid blood flows are used as points of reference. Laying these off on the records of ventilation and circulation we can consider the details of one cycle of this periodicity from *2′* to *3′*. Respiratory movements increase in amplitude during the decrease in blood flow. This augmentation then suddenly gives way to apnea, also during the decreasing blood flow. This apnea is then continued throughout the period of increased flow of blood from *3* to *3′* and even into the early part of the next period of decreased flow. These findings hold essentially in each periodic group.

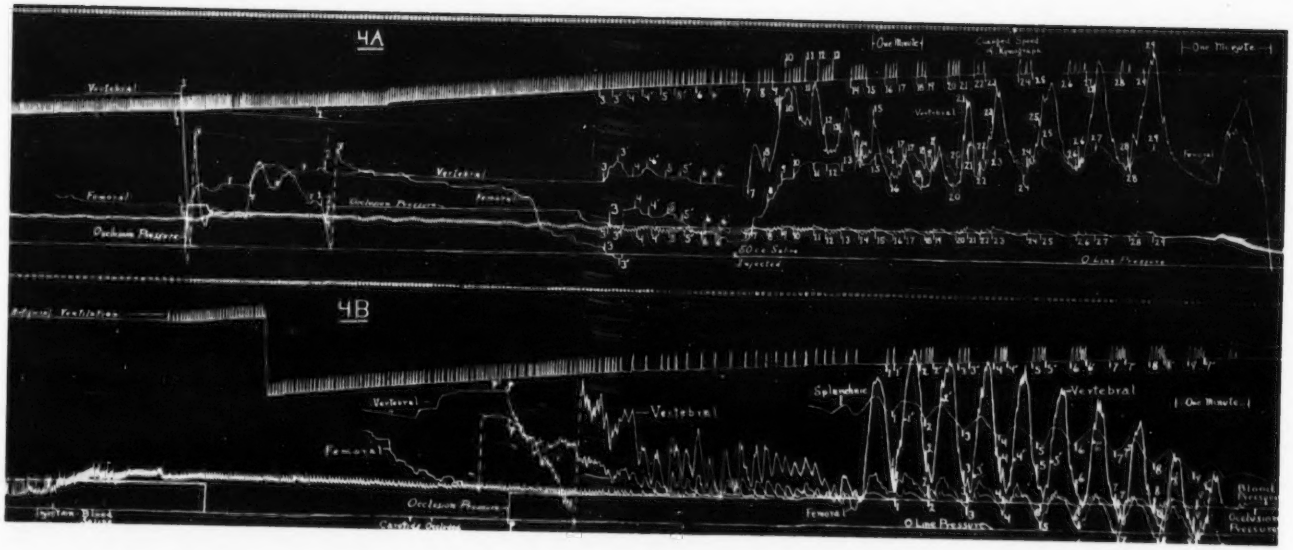
The carotid blood flow and the submaxillary venous flow follow, in general, the changes in mean blood pressure. This is not true of the heart rate. During the increase in blood pressure, heart rate and blood pressure run parallel, but heart rate continued to rise after mean blood pressure has started to fall, and diminishes again before mean blood pressure has reached its lowest level. At *3′*, blood pressure has reached its maximum, but heart rate continues to increase. The relation of heart rate to mean blood pressure is similar to that noted in figure 2B.

In figure 3, the onset of the respiratory movements of each respiratory group occurs during the period of decreased blood pressure and carotid blood flow agreeing with the results in figure 2B. The time relation of the development of apnea, however, was somewhat different. In figure 3 apnea begins towards the end of decreased carotid flow, whereas in figure 2B, apnea begins during the early part of the subsequent increased flow.

It is accordingly pointed out that the apneas of 2B were associated with a recovery process. This type of apnea may, therefore, be termed a recovery apnea. In contrast, the apneas of figure 3 are associated with a depression process as they occur in general during the decreased flow of blood. These may be designated as depression apneas. Although the onset of the apneas appear in general during the decreased blood flows, slight variations occur at *4* and *8*. The respiratory movement at *4* occurs late, that is, after the completion of the main body of respiratory movements. This may have its explanation in the observation that the last respiratory movement of each respiratory group shows a tendency to lag (see fig. 2B also). For example, note the respiratory group *1′* to *2*. The interval just to the left of *4* may, therefore, possibly be regarded as a reduction in rate of respiratory movements. The respiratory group *7′* to *8* represents a different variation. By extending the rate of respiration of this group it will be seen that the increased flow of blood at *8* begins before the point at which another (hypothetical) respiratory movement would take place. Accordingly the apnea to the right of *8* could be looked upon as a recovery apnea. Thus the increased flow may have prevented this hypothetical respiration. On the other hand, the increased flow at *8* following so closely upon the respiratory group indicates that a depression apnea may have actually started at the moment of the increased flow of blood. Thus depression and recovery apnea may indistinguishably shade into each other. However, conditions may occasionally arise depending upon the prevailing circumstances which would interpose a single respiratory movement between a depression and recovery apnea. Such conditions possibly obtain in regard to the initiating respiratory movement at *1′*.

Returning to a comparison of figure 3 and 2B, if we add one hypothetical respiratory movement to cycle *7′* to *8*, there is closer resemblance between figures 2B and 3. With this addition, apnea would also develop during the increasing flow of blood and could, therefore, be looked upon as a recovery apnea similar to those occurring in figure 2B. It is thus suggested that the conditions in figures 2B and 3 may be more similar than the records indicate and that the suddenness of chemical changes brings about more apparent rather than real differences.

Figures 4A and 4B are continuous records of another experiment. The procedure used in obtaining figures 4A and 4B was the third method. The central end of the left vertebral artery was connected to the peripheral end of the right vertebral artery through a volume flow thermopile and a pressure cuff applied to permit graded occlusion. Previous to figure 4A the carotids were occluded, thus leaving one vertebral artery and the spinal arteries to supply blood to the brain. The continuous thermoelectric method of recording was used for the vertebral and femoral blood flows. Femoral blood pressure, respiration, occlusion pressure, blood flows, and


4A
Vertebral
Femoral
Occlusion Pressure
Vertebral
Femoral
Occlusion Pressure
One Minute
Control Speed of Kymograph
One Minute
Vertebral
Femoral
60 cc Saline Injected
O line Pressure
4B
Vertebral
Femoral
Vertebral
Splanchnic
Vertebral
One Minute
Occlusion Pressure
Femoral
O line Pressure

Figs. 4A, 4B

time in seconds were simultaneously recorded. Dotted lines indicate the raising of the writing points to keep them on the record. The anesthetic used was amytal.

Occlusion pressure on the vertebral artery was increased at *1* to a point considerably above mean blood pressure. There was temporary stimulation of respiratory movements which gave way to a very short apnea just preceding the increased vertebral flow at about the middle of the period *1* to *2*. This increase in vertebral flow is associated with an increase of mean blood pressure and is followed by a second increase accompanied by another increase in blood pressure. At *2* the occlusion pressure was raised considerably above mean blood pressure resulting in a complete obstruction of the vertebral artery. During the period *2* to *3* blood flow reached the brain by way of the spinal arteries only. Respiratory movements were maintained at a slower rate and greater amplitude than prior to *1* where vertebral blood flow was copious. At *3* the occlusion pressure was lowered and vertebral blood flow was reëstablished when blood pressure increased sufficiently to overcome the occlusion pressure. The waves in mean blood pressure are reflected in the vertebral flow record.[3] The periodicity in ventilation was rather indefinite which would correspond to the relatively small changes in volume flow of blood. At number *7* the center was depressed and responded well to the subsequent larger variations in blood flow. At the end of *4A* artificial ventilation and saline solution were administered to resuscitate the animal. A second series of responses similar to those shown in *4A* was then elicited by a subsequent reduction in volume flow of blood.

Partial occlusion of the vertebral artery at *P* brought about a marked decrease in the vertebral blood flow. There was temporary stimulation of respiratory movements which gave way to gradual slowing of the rate, accompanied by a slight increase in amplitude. With each respiratory movement there was a change in vertebral blood flow which was, presumably, secondary to the respiratory movements. Mean blood pressure gradually decreased until it was almost equal to the occlusion pressure. This brought about a further decrease in vertebral blood flow, when suddenly periodic fluctuations in mean blood pressure and vertebral flow of blood appeared. Apnea develops with the first increase in vertebral blood flow and mean blood pressure. These large circulatory changes can hardly be attributed to a respiratory movement. Since the initial large increase in vertebral blood flow had its onset during the last respiratory

[3] Attention is called to the increase of vertebral blood flow occurring slightly later than the increase of the femoral and splanchnic flows as this lack of synchronism might be misinterpreted. It is probably due to the fact that the occlusion pressure applied to the vertebral artery does not allow an increase of blood through the artery until the arterial blood pressure is sufficient to overcome the occlusion pressure.

movement preceding the initial apnea, this apnea could be regarded as secondary to improved circulation. It is, therefore, considered as a recovery apnea. This recovery apnea marks the establishment of periodicity.

The points of reference used in analyzing figure 4A and 4B are different than those used in the previous figures. The beginning and end of each respiratory group is set off on the vertebral blood flow and mean blood pressure records and only the beginning of the respiratory group is marked off on the femoral, splanchnic, and blood pressure records. (The splanchnic venous blood flow was followed by the external drop method.) The onset of respiratory movements during periodicity occurred, as in the previous figures, after a period of decreased blood pressure and decreased blood flow, and continued into the increased blood pressure and increased blood flows. In this respect the results are similar to figure 2B Respiration was initiated on the average about 11 seconds after the onset of the decreased blood flows. In figure 3 respiration began about 25 seconds after the decreased blood flows was initiated and in figure 2B it began more than a minute later. The periodic changes in circulation occur abruptly and are of extremely short duration, but at the crests and troughs, blood flow changes occur more slowly. These high and low flows are, therefore, of relatively long duration. Assuming a good recovery during the high blood flows there is little time for altered chemical changes during the rapidly decreasing flow. Furthermore, the center may now be in a condition to withstand a short period of decreased flow without responding with marked stimulation. Agreeing with this we find that the first respiration occurs in the trough of the volume flow record during which the respiratory center presumably continues to be stimulated. Blood volume flow now suddenly increases, but again the period of increase is too short to compensate the previous lack of blood and thus occasionally the last respiration may occur well up on the ascending limb of the volume flow record. If we draw a line of mean blood volume flow across the record it may be stated as a gross approximation, that on the whole respiratory movements occur during lowered blood flows and pressures and that apneas occur during increased blood flows and pressures.

Heart rate did not show periodicity in figure 4B. This constancy of rate, indicating lack of change of vagal tone was common in all experiments in which amytal was used as the anesthetic. Even with increased intracranial pressure cardiac slowing was lacking when amytal was used. This is in contrast to the marked changes in heart rate in figure 3 in which urethane and morphine was the anesthetic. This constancy of heart rate, together with the marked changes in mean blood pressure suggest periodicity of the vasomotor center. It was hoped that direct evidence of active periodic vasoconstriction and dilatation would be obtained by recording the various blood flows throughout the series. As seen in figure 4B, the splanchnic and

femoral blood flows follow the changes in mean blood pressure. The results, however, do not necessarily prove that active vasomotor changes are absent. It is possible that the increased driving head of pressure is accompanied by either an active vasoconstriction or vasodilatation. Experiments using a constant perfusion pressure through tissue isolated from the periodic circulation are desirable.

Discussion. In the preceding paper it was pointed out that the periodic changes in ventilation produced by moderate obstruction of cerebral blood vessels might be a resultant of two circulatory changes which are occurring periodically and at the same time—namely, changing cerebral flow of blood and changing mean blood pressure. The same possibility holds for the periodic ventilation produced by greater obstruction of the cerebral blood vessels. Data bearing on the relative importance of these two factors are therefore briefly considered.

Considering the central chemical effects of altered flow of blood first, it is believed that normally the respiratory center is provided with an abundant supply of well oxygenated blood and under these conditions is protected against gross and rapid chemical changes, but that with impaired circulation conditions are provided which are susceptible to gross fluctuations which in turn lead to periodicity of circulatory and respiratory functions. Thus when blood flow is sufficiently diminished oxidations are impaired and anaerobic conditions develop. Under these conditions lactic acid accumulates in the brain at a rapid rate (McGinty and Gesell, 1925). There is thus a sudden increase in fixed acids, a sudden liberation of carbonic acid from the tissue bases, and a reduction in the tissue buffer base. These changes are accompanied by a decrease in transport of acid from the tissues. Not only is there a decrease in the physical transport of acid but with the failure of the tissues to get oxygen from the blood there is a broken coördination of the dual function of hemoglobin with a consequent impairment of chemical transport of acids from the tissues. The increase in acidity of the tissues impairs oxidations still more. There is thus a sudden impairment of oxidation and an increase in acidity. The cardio-inhibitory center is markedly stimulated during decreasing volume flow of blood and so is the respiratory center. Both of these conditions lead to a marked increase in ventilation of the blood which raises the oxygenation and lowers the carbon dioxide tension of the blood. Suddenly this highly ventilated blood flows through the respiratory center at an augmented rate and the chemical processes are as quickly reversed. Increased supply of oxygen and high concentration of oxidizable materials lead to supernormal oxidations. Lactic acid is removed by oxidation and by shift to the precursor state (Hill, 1913; Meyerhof, 1921). Base is liberated to combine with free carbon dioxide. The transport of acid is improved by increase in flow of blood, by the lowered carbon dioxide tension of the blood and by

the reëstablishment of the coördination of the dual function of hemoglobin. Apnea comes on and lasts until chemical stimulation is reëstablished.

The changes in periodicity of ventilation may also have been modified by the chemical changes occurring in various parts of the reflex arc for the volume flow of the body as a whole appears to be periodic. The work of Glazer (1927), Winkler (1929), and Gay (1929), who showed the effects of hemorrhage, cyanide and low oxygen mixtures on the response of the striated muscle to reflex, motor nerve and direct stimulation suggest chemical influences outside the respiratory center proper.

To what extent the periodic changes in ventilation and circulation are influenced by vascular reflexes set up by changes in mean blood pressure is difficult to state with certainty. With regard to the heart reflex the findings are more positive. A fall in pressure which should reflexly establish an increase in heart rate is accompanied in our experiments by a marked slowing of the pulse. Thus if the vascular reflex is operating it is relatively weak as compared with the central chemical stimulation of the inhibitory center. But the fact that the central chemical and vascular reflex effects are theoretically additive in the case of respiration offers difficulty in evaluating the quantitative effects of each. Reasoning by inference from the results on heart rate it might be concluded that here too the central chemical effect is the important effect. The difficulty of obtaining periodic respiration, for the present at least, precludes the use of controls in which the vagus nerves are cut.

If the changes in blood flow are the initiating cause of periodic changes in ventilation, as they appear to be in the present experiments, we must attempt to explain periodicity in blood flow. It is only necessary to accept the view (Gesell, 1929) that the metabolism of the circulatory centers is a controlling factor in the regulation of circulation and by a chemical mechanism similar to that controlling ventilation. It is thus assumed that the chemical changes just described for the respiratory center occur within the circulatory centers as well. Experiments 2A, 2B, and 3 show a fall in mean blood pressure associated with a decrease in heart rate, and an increase in blood pressure associated with an increase in heart rate. Thus the cardio-inhibitory and respiratory centers show similar and simultaneous changes in activity.

Experiments in which amytal was used as the anesthetic, marked periodicity occurred without significant changes in heart rate. These experiments thus indicate that periodicity of the vasomotor center may bring about periodicity in mean blood pressure. Since respiratory and circulatory stimulation appear simultaneously with decreasing blood flow, a common stimulating mechanism through increased acidity and impaired oxidations is supported. As for depression of respiratory movements which may follow abruptly on stimulation during decreased flow, it is

pointed out that there can hardly be a more effective mechanism for bringing about complete depression of a nerve center than an impaired supply of oxygen and impaired removal of acid both of which lead to increased acidity which in turn impairs oxidations still more. Thus it is not surprising that when the respiratory center is stimulated to its maximum while the energy of the center is being rapidly depleted, that stimulation should promptly give way to depression. Obviously the resistance of centers must vary and for that reason stimulation of the respiratory center of one animal may carry through the entire period of decreased blood flow and into the next period of increased flow, whereas another may give way during the period of decreased flow. Thus apnea which develops with decreased flow although of different immediate origin is perhaps not so different from the apnea initiated during increasing blood flow. For that reason it may not be profitable to attempt a division of periodic ventilation into distinct types unless it be to divide it into periodic respiration with and without distinct apneas.

SUMMARY

Periodic respiration with pronounced apneas, produced by marked occlusion of cerebral blood vessels, was studied.

The blood flow to the brain was curtailed by three methods; 1, ligation of spinal arteries plus the application of occlusion pressure to one cerebral artery and occlusion of remaining cerebral arteries; 2, increased intracranial pressure, and 3, application of occlusion pressure to one of the four cerebral arteries with the occlusion of the others.

Increasing curtailment of blood flows was accompanied by initial stimulation of respiratory movements, which was followed by a period of partial or complete depression. After this depression, periodicity of ventilation sometimes followed.

The periodicity of ventilation thus established was accompanied by periodic changes in mean blood pressure, volume flow of blood, and heart rate. The respiratory groups were initiated during decreasing flow of blood and decreasing blood pressure. In some experiments they terminated in apnea while blood pressure and blood flow were still decreasing and in others they extended for a short period into the increasing blood pressure and increasing blood flow.

Since apnea was initiated towards the close of decreasing blood flow or beginning of increasing blood flow, it was suggested that apnea was produced by both severe depression and by processes of recovery. Accordingly apneas were designated as depression and recovery apneas.

In experiments in which periodic changes in heart rate occurred, slowing of the heart was in the main associated with decreasing mean blood pressure and decreasing flow of blood, and augmentation with increasing blood

pressure and increasing flow of blood. It is thus suggested that the cardio-inhibitory center is stimulated like the respiratory center by the same chemical mechanism.

It was suggested that the rapid rate and the gross change in chemical state of the medullary centers coupled with impaired functional activity of these centers provide conditions for periodic activity. A decreased blood flow brings about a decrease in the chemical and physical transport of acid, a decrease in oxidations with a consequent broken coördination of the dual function of hemoglobin all resulting in an altered state of oxidations, and increased acidity of the respiratory center and an over ventilation of the blood. An increased blood flow of oxygenated blood now reverses these processes. Oxidative and non-oxidative removal of lactic acid and the liberation of free base and improved transport of acid combine to bring about a new state of oxidation and a decrease in acidity.

The absence of changes in heart rate in one series of experiments with marked periodicity of ventilation, blood pressures, and blood volume flows indicates that periodicity may have its origin in periodic changes of the vasomotor center. However, the volume flow of blood ran parallel in the splanchnic, femoral, submaxillary, and cerebral blood vessels. Direct evidence of active vasomotor changes was, therefore, not obtained.

Our results suggest that periodic chemical stimulation of the circulatory centers produces periodic changes in ventilation of a central chemical origin. Since decreasing blood pressure may reflexly stimulate pulmonary ventilation this phenomenon may be looked upon as a possible modifying factor.

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THE EFFECT OF VARIOUS ANTERIOR PITUITARY PREPARATIONS UPON BASAL METABOLISM IN PARTIALLY THYROIDECTOMIZED AND IN COMPLETELY THYROIDECTOMIZED GUINEA PIGS

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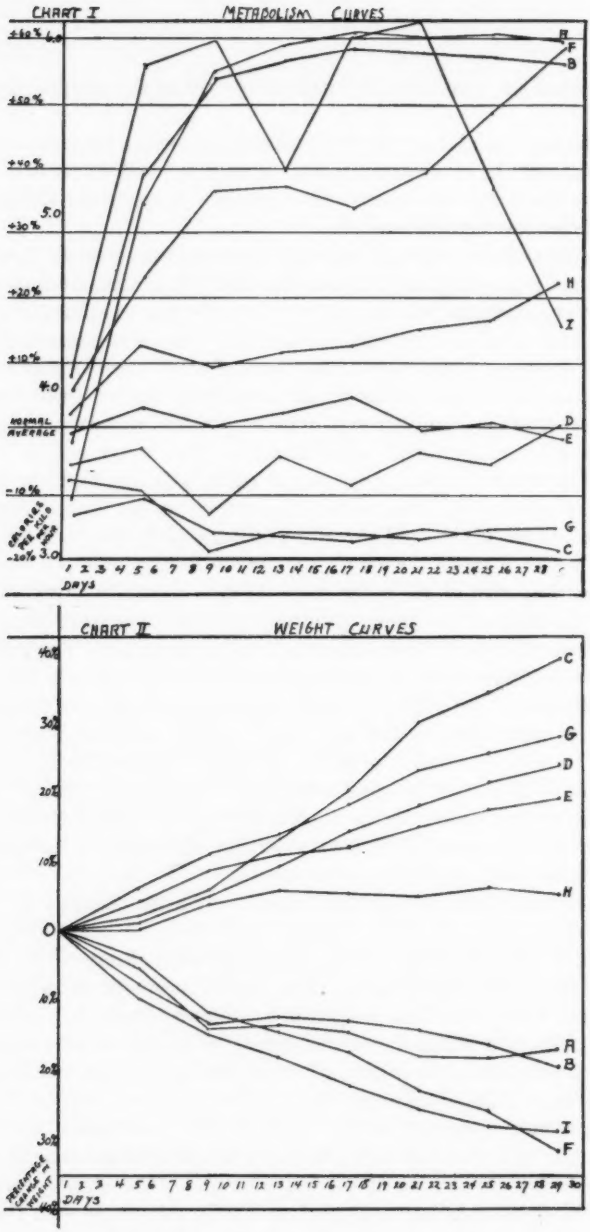
Loeb (1), Loeb and Kaplan (2), McCordock (3), and later Loeb and Bassett (4) and Silberberg (5) studied the effect of various anterior pituitary preparations upon the thyroid gland in guinea pigs. It was found in these experiments that various preparations had different effects. Subcutaneous injections of acid extracts of fresh cattle glands caused a very marked hypertrophy of the thyroid gland in a very short time, while, on the contrary, the feeding of Armour's pituitary tablets produced structural changes indicating a decreased activity of the gland. In view of the effect of these preparations on the thyroid gland and considering the significance of the thyroid gland in basal metabolism we (6) then studied the action of these anterior pituitary preparations on the basal metabolism in guinea pigs. In these experiments (6) it was found that although both preparations caused a marked rise in the basal metabolic rate, they differed in the degree and the sharpness of the rise which they caused, and in the character of the curves following the period when they had reached the maximum point. The experiments of Loeb upon the effects of these anterior pituitary preparations on the structure of the thyroid gland suggested that in all probability the acid extract of anterior pituitary substance would affect the basal metabolism through changes which it produced in the thyroid gland, while the Armour preparation would affect the metabolism independently of such changes. As stated, the experiments of Loeb (1), (2), (4) and McCordock (3) had proven that Armour's anterior pituitary preparation decreased the activity of the thyroid. If then the administration of anterior pituitary (Armour) caused a marked rise in basal metabolism, this effect could not be due to the mediation of the thyroid gland. On the other hand, the fact that extracts of anterior pituitary prepared in our laboratory was shown to have a markedly stimulating effect on the thyroid gland made plausible the assumption that the effect of this substance on basal metabolism was by

way of the thyroid gland. If this reasoning is correct then thyroidectomy should prevent the rise in metabolism caused by acid extracts of anterior pituitary, while the rise caused by Armour's preparation should be left undiminished by thyroidectomy.

We tested this interpretation in the following experiments: Twenty-four male guinea pigs, weighing between 350 and 500 grams, were used. Ten of these were as completely thyroidectomized as was possible; four of these ten were then given daily injections of 1 cc. of the acid extract of anterior pituitary substance, and four others were given 5 grain Armour anterior pituitary tablets by mouth daily; the remaining two of these ten guinea pigs were used as completely thyroidectomized controls, to which no pituitary preparations were administered. Ten other guinea pigs were partially thyroidectomized, one and two-thirds to one and one-half lobes of the thyroid having been removed previous to the daily subcutaneous injection of 1 cc. of the acid anterior pituitary extract to four animals and the feeding of 5 grain Armour's anterior pituitary pills to four others. The remaining two of these ten were used as partially thyroidectomized controls to which no anterior pituitary preparations were administered. Four guinea pigs were used as normal control animals. The basal metabolism determinations were then made on each of the twenty-four animals every fourth day with the Haldane open circuit respiratory calorimeter. Each observation consisted of a two-hour period on this apparatus. The animals were starved for a period of 24 hours before the determinations were made. The injections and feedings of the anterior pituitary preparations were made previous to the starvation period. The experiments were continued for a period of 28 days after the beginning of the administration of the anterior pituitary preparations.

The results of these experiments are shown on the charts. The number of calories per kilo per hour together with the percentage rise or fall in metabolism are recorded on the perpendicular lines. The days are recorded on the horizontal lines. The curves represent the averages of all the animals which had been subjected to a certain experimental procedure.

Chart I gives a summary of the results of the above described experiments together with the results of some previously reported experiments (6) upon the effects of acid anterior pituitary extracts and of the Armour 5 grain anterior pituitary tablets upon the basal metabolic rate in normal guinea pigs. Chart II gives a summary of the average weight curves of animals during the course of the experiments. The charts are arranged so that the determinations recorded on the first day represent the average basal metabolism of a given series previous to the administration of the anterior pituitary preparations over preliminary trial periods of one to two weeks. The effects of the anterior pituitary preparations upon the metabolism as well as the control values are recorded in subsequent 4 day



Charts I and II. *A* = Complete thyroidectomy; given Armour anterior pituitary tablets. *B* = Partial thyroidectomy; given Armour anterior pituitary tablets. *C* = Complete thyroidectomy; controls. *D* = Partial thyroidectomy; controls. *E* = Normal controls. *F* = Normal animals given Armour anterior pituitary tablets. *G* = Complete thyroidectomy; given acid extract of anterior pituitary substance. *H* = Partial thyroidectomy; given acid extract of anterior pituitary substance. *I* = Normal animals; given acid extract of anterior pituitary substance.

periods, each value being the result of the average of all the animals used. Curves *C*, *D* and *E* on chart I represent the data relative to the respiratory exchange measurements in the control animals, to which no anterior pituitary preparations had been administered. Curve *E* represents the curve obtained in the normal guinea pigs. The mean average metabolic rate for the 4 normal control guinea pigs is 3.75 calories per kilo per hour. This value corresponds very well with the results of a much larger series of control animals which we had previously reported (6) and it was, therefore, thought to be a sufficient control series for our purpose in this experiment. It will be noticed on curve *E* on chart II that there is an average gain in weight in the animals of this series in the twenty-eight day period of approximately 17 per cent. All the control animals gained weight at approximately the same rate.

Curve *C* represents the values of the two completely thyroidectomized guinea pigs to which no anterior pituitary preparation had been administered. Beginning on the fourth to the seventh day following the thyroidectomy the average metabolic rate of the two guinea pigs is approximately 5 per cent lower than in the normal control animals and then gradually decreases to a level approximately 17 per cent below the normal level where it remains throughout the 28 day period. There is a tendency for the individual animals as well as the average curves to show an increasingly lower metabolic rate at the end of the 28 day periods.

Chart II shows the average gain in weight of these animals to be approximately 40 per cent within the 28 day period, which represents an increase in rate of gain over the normal control animals of 23 per cent.

Curve *D* on chart I represents the average values of the respiratory exchange of two partially thyroidectomized guinea pigs. The curve of the metabolic rate is slightly lower than the very lowest limits of the values obtained for normal guinea pigs. There is a tendency for the metabolic rate to return to the normal level toward the end of the twenty-eight day periods, probably as the result of compensatory hypertrophy of remaining thyroid gland tissue. The average weight curve as well as the individual weight curves show these animals to gain weight at a slightly more rapid rate than do the normal control animals, but not as rapidly as do the completely thyroidectomized guinea pigs.

Curve *I* on chart I represents the curve of metabolism obtained for 8 normal guinea pigs which had received daily injections of an acid anterior pituitary extract. This series was reported in a previous communication (6) and was inserted here in order that the effects of this pituitary preparation upon normal animals may be compared with its effects upon partially and completely thyroidectomized guinea pigs. In normal animals the daily subcutaneous injections of acid extracts of anterior pituitary glands of cattle cause a very marked and rapid rise in basal metabolism, which

reaches a maximum of approximately +60 per cent within the first 10 days. After this maximum rise, the basal metabolism gradually returns to a level approximately 15 per cent higher than the average found in normal animals. This rise in metabolism is accompanied by a rapid and marked loss in weight of aproximately 30 per cent, as shown in curve *I* of chart II.

Curve *F* on chart I shows the effect of the daily feeding of 5 grain Armour anterior pituitary tablets to 8 normal guinea pigs. This series was also previously reported (6). This anterior pituitary preparation in normal animals causes a gradual steady rise in basal metabolism which reaches a maximum of approximately +60 per cent in about 30 days. There seems to be a cumulative effect in this case and there is no tendency on the part of the metabolism to return to a lower level subsequently throughout the period of our investigation. Chart II shows the weight loss to be not quite as rapid at first as in the normal guinea pigs which had received the acid extract of anterior pituitary substance. However at the end of the 28 day period both groups of animals had lost approximately the same percentage of weight; the maximum average loss in weight amounted to approximately 30 per cent of the original weights of the animals.

Curve *A* on chart I shows the effect upon metabolism of the daily administration of 5 grain Armour anterior pituitary tablets given to four completely thyroidectomized guinea pigs. There is a rather rapid and steady rise in basal metabolism which reaches a maximum of approximately +60 per cent in about 30 days. Although this rise is somewhat more rapid than that seen in the normal animals to which the same drug had been administered, the weight loss is somewhat less in the former series, as shown by comparing curves *A* and *F* in chart II. The curve of basal metabolism obtained in this series is similar to the curve of the series in which the normal animals were fed with Armour's anterior pituitary preparation in that neither series shows a tendency on the part of the metabolism to return to a lower level subsequently throughout the period of our investigation.

Curve *B* on chart I shows the effect of the daily feeding of one 5 grain Armour anterior pituitary tablet to partially thyroidectomized guinea pigs. The curve of the basal metabolism parallels very closely the curve obtained in the above series in which the Armour preparation had been fed to completely thyroidectomized guinea pigs. The weight curves are also approximately identical. These two series together show that there is no difference in the effects of the Armour anterior pituitary upon basal metabolism in the completely thyroidectomized guinea pigs, as compared with the effects of this preparation in partially thyroidectomized guinea pigs.

Curve *G* of chart I represents the curve of respiratory exchange measure-

ments upon 4 completely thyroidectomized guinea pigs which had received daily 1 cc. injections of acid anterior pituitary extract. This curve parallels very closely the curve obtained in the control series of guinea pigs which had been completely thyroidectomized but to which no anterior pituitary preparation had been administered, and shows a lowered basal metabolic rate which reaches a maximum of minus 17.5 per cent. The average gain in weight of the animals of this series is considerably greater than that which occurs in the normal control animals but is not quite as rapid as the average gain in weight of the completely thyroidectomized controls. It appears from these results that the acid extract of cattle anterior pituitary glands is entirely inefficient in causing a rise in basal metabolism in completely thyroidectomized guinea pigs, although as shown above it causes a very marked and rapid rise in metabolism in normal animals. The curves of the individual animals in the former series show no important variations from the average curve.

Curve *H* of chart I shows the effect of daily 1 cc. injections of acid extracts from cattle anterior pituitary gland upon the basal metabolism in 4 guinea pigs which had one and two-thirds of the thyroid gland removed previous to the administration of the anterior pituitary preparation. The basal metabolism of this series ranges from approximately 10 per cent to 20 per cent higher than the average found in normal guinea pigs. There is in all the individual animals as well as in the average results a tendency for the basal metabolism to rise gradually to the higher level toward the end of the period of our investigation. The average weight curve of the guinea pigs in this series shows that the weights remain approximately stationary throughout the experiment with very slight tendency on the part of the animals either to gain or lose weight. It appears from this series that the acid extract from cattle anterior pituitary glands is effective in causing a rise in metabolism which rise apparently is in proportion to the amount of thyroid gland tissue present in the guinea pig. However, further experiments will be necessary to show to what extent this quantitative relationship holds true.

We may therefore conclude 1, that Armour anterior pituitary tablets cause a marked rise in basal metabolism in guinea pigs. This rise in metabolism occurs similarly after partial or complete thyroidectomy and is therefore independent of changes in the thyroid gland. 2. The daily injections of 1 cc. of an acid extract from cattle anterior pituitary glands also cause a marked and rapid rise in basal metabolism in guinea pigs. The rise in basal metabolism caused by these injections, in contrast to the effects of administration of Armour anterior pituitary tablets, does not occur after complete thyroidectomy and is apparently entirely dependent upon hypertrophic changes which it produces in the thyroid gland. 3. The daily injections of 1 cc. of the acid extract of cattle anterior pituitary

glands in partially thyroidectomized guinea pigs causes a slight but definite rise in basal metabolism.

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DEPLETION OF MUSCLE SUGAR BY ADRENALIN. II

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In an earlier paper (Bischoff and Long, 1930) the depletion of muscle sugar by adrenalin was reported. Following a subcutaneous injection of 0.25 mgm. or more per kilo of adrenalin to the rabbit, the muscle sugar fell below the normal resting level and remained low from the eighth to the twenty-fourth hour. At the third hour the muscle sugar had already fallen to the fasting value, while the blood sugar was at the peak of the curve.

No attempt was made at that time to account for the observation, though the simultaneous changes in liver and muscle glycogen, blood lactic acid, and blood sugar were well established. Cori and Cori (1930) have since established the maximum rate of absorption of adrenalin from subcutaneous depots by their work on continuous intravenous injections of adrenalin. If their maximum absorption time of four hours is accepted, the depletion of the muscle sugar must occur during the recovery period.

OBJECT. In the present paper we have attempted to investigate the mechanism by which the muscle sugar may be depleted through the action of adrenalin. The continuous intravenous injection method was used so that the time of the adrenalin activity was accurately established. The minimum subcutaneous and continuous intravenous doses of adrenalin which would lower the muscle sugar were established. Muscle sugar, muscle glycogen, hydrolyzable muscle sugar and blood sugar were determined during both the active and the recovery periods.

EXPERIMENTAL PROCEDURE. Rabbits which had been fasted 24 hours were used and litter mates were selected as controls. The analytical methods for determination of blood sugar, blood lactic acid, and muscle glycogen and sugar were the same as described in earlier publications (Bischoff and Long, 1930). Parke, Davis & Co. adrenalin hydrochloride diluted in normal saline was used for continuous intravenous injection. This solution was injected into the marginal ear vein from a burette arrangement.

EXPERIMENTAL RESULTS. *Minimum subcutaneous dose.* In our original studies 0.25 mgm. or more adrenalin per kilo was injected subcutaneously to bring about a fall in muscle sugar. Since these studies were made an instance was found in which 0.25 mgm. per kilo did not produce a low

muscle sugar at the ninth hour, although the blood sugar had risen to 348 the third hour. The effect of 0.06 mgm. and 0.10 mgm. per kilo was tried in eight series of the present experiments. The rabbits were killed 1.5, 3, 5, 7, and 8 hours after dosing. Resting muscle sugar values were obtained in all these experiments with the exception of two series. At five and eight hours after injection, 0.06 mgm. adrenalin produced values of 76, 75, 69, and 70, which were slightly lower than the control values. The average resting muscle sugar value for sixteen rabbits, fasted 24 hours, obtained by us is 91 mgm. per 100 grams with extreme values of 109 and 76. Apparently the minimum dose which will show a change in muscle sugar is 0.06 mgm. per kilo of adrenalin given subcutaneously.

Minimum continuous intravenous dose. A 90-minute continuous intravenous injection of 0.04 mgm. adrenalin per kilo hour (total, 0.06 mgm.per kilo) produced no lowering of the muscle sugar at the fourth hour. A three-hour injection of 0.022 mgm. per kilo hour (total, 0.07 mgm. per kilo) and a three-hour injection of 0.06 mgm. per kilo hour (total, 0.18 mgm. per kilo) produced no lowering of muscle sugar the seventh and eighth hours. The minimum dose which produced a low muscle sugar was 0.15 mgm. per kilo, given in a three-hour injection at the rate of 0.05 mgm. per kilo hour. Considering the natural variation in tolerance to adrenalin between different animals there would appear to be no difference between the effective dose which will lower the muscle sugar, whether intravenous or subcutaneous.

Intravenous and subcutaneous injections. Cori and Cori and Buchwald (1930) showed that when adrenalin is given in a continuous uniform injection at the rate of 0.03 to 0.06 mgm. per kilo hour a rise in blood sugar and blood lactic acid takes place similar to the rise of these blood constituents produced by the subcutaneous injection of 0.20 mgm. adrenalin per kilo. "Since after the subcutaneous injection of 0.2 mgm. of adrenalin per kilo the hyperglycemia persists for four hours and since adrenalin produces hyperglycemia only so long as it enters the blood stream, the absorption of adrenalin from its subcutaneous depot must have persisted for at least four hours." In the experiments of Cori and Cori the continuous intravenous injection of adrenalin was made for 120 minutes. In our experiments the injection period was extended as long as seven hours. Our experiments show that after the fourth hour the blood sugar curve falls even though the injection of adrenalin is continued at the same rate. The fall is not as rapid as when the adrenalin injection is stopped. The curve follows almost exactly the subcutaneous injection of the same amount of adrenalin. Our data show that Cori and Cori were conservative in establishing four hours as the maximum time for subcutaneous absorption. They pointed out that since there is no rise in blood pressure with 0.20 mgm. per kilo of adrenalin given subcutaneously, the rate of absorption must be less than their four-hour calculation would indicate, and suggested a pos-

sible but improbable rapid destruction of adrenalin in the subcutaneous tissue. By following the injection over a longer period we have reconciled these apparent anomalies. The subcutaneous injection of adrenalin is analogous to a continuous intravenous injection of the same amount of adrenalin over a six-hour period. Typical blood sugar curves following the continuous intravenous injection of adrenalin are given in figure 1. The shape of the curve is characteristic. Because of the natural variation in tolerance of different animals to adrenalin the height of the sugar curve

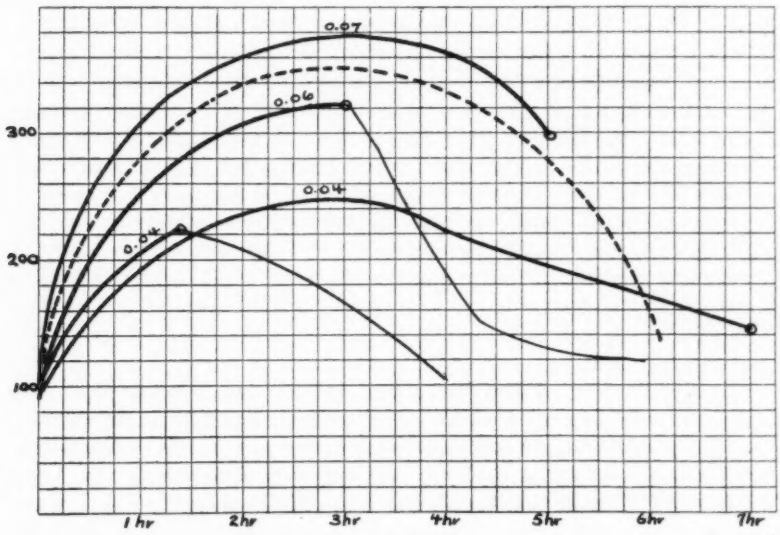


Fig. 1. *A comparison of the blood sugar curves following continuous intravenous and subcutaneous doses of adrenalin.* The heavy solid lines chart the blood sugar during the period of continuous intravenous injection terminated by the circle. The light solid lines chart the recovery period. The dotted line represents a typical curve following a subcutaneous injection. The figures over the curve give the injection rate of adrenalin in milligrams per kilo hour.

varies considerably, 0.03 mgm. per kilo hour in one animal producing a higher curve than 0.07 mgm. in another animal. The contrary inference might be deduced from the chart, in which only a few of many curves were plotted to avoid confusion.

Diminished tolerance for adrenalin. The descending blood sugar curve beginning at the fourth hour during a continuous intravenous injection of adrenalin at a uniform rate indicates a development of tolerance by the animal during the course of the injection. In two experiments the rate of injection was doubled after the blood sugar curve was descending, when

the blood sugar curve again rose. (See table expts. 12 and 13.) The fall in the blood sugar curve during a continuous intravenous injection of adrenalin cannot therefore be attributed to a depletion of the stores at the source of the blood sugar.

Low muscle sugar during adrenalin action. The lowering of the muscle sugar to subnormal values does not take place during the period when adrenalin is accelerating the breakdown of muscle glycogen and retarding the utilization of glucose by the muscle. The experiments with subcutaneous injections showed normal muscle sugar values at the third hour, a time at which the subcutaneously injected adrenalin must be conceded to be not yet entirely diffused from its subcutaneous depot. Five determinations of muscle sugars were made in the present series for animals which had continuous intravenous injections of adrenalin for from three to seven hours without allowing for a recovery period. The injection of 0.09 mgm. adrenalin per kilo hour for three hours produced a high muscle sugar the third hour. Two injections at the rate of 0.03 mgm. per kilo hour for six hours produced normal muscle sugar values the sixth hour, as did 0.04 mgm. per kilo hour for five hours followed by 0.10 mgm. for one hour. A seven-hour injection of 0.04 mgm. per kilo hour produced a low muscle sugar. The blood sugar in this experiment at the seventh hour had descended from a high value to 145 so that the adrenalin had apparently ceased to be effective.

Blood lactic acid. Blood lactic acid values were determined in two experiments. Cori and Cori and Buchwald showed rather conclusively that the blood lactic acid and blood sugar curves run parallel for a 120-minute period of adrenalin infusion. In our two experiments the blood lactic acid fell before the blood sugar. At an injection rate of 0.05 mgm. adrenalin per kilo hour, the blood sugar was 282 the second hour, the blood lactic acid 145. One hour later the blood sugar was the same, the lactic acid having fallen to 102. The injection, which lasted three hours, was stopped at this time. The following hour the blood sugar had descended to 196, the lactic acid to 63. The fifth hour both blood sugar and lactic acid were normal, 105 and 23 respectively. This experiment will be found as number 3 in table 1. In the second lactic acid experiment 0.09 mgm. per kilo adrenalin was injected for three hours. The second hour the blood sugar was 308, the blood lactic acid 126. The third hour the blood sugar had risen to 328, the lactic acid fallen to 68.

Muscle glycogen. Muscle glycogen values were obtained simultaneously with muscle sugar values for twelve rabbits which had received adrenalin under various conditions. In all instances (5) in which low muscle sugar values were found low glycogen values (33 to 104) were also found. Low glycogen values, however, were observed in five cases in which the muscle sugar values were normal or high. The fall in muscle sugar cannot, there-

TABLE 1

Effect of the continuous intravenous injection of adrenalin upon the muscle carbohydrate

EXPERIMENT NUMBER	DOSE PER KILO HOUR	INJECTION PERIOD	TOTAL DOSE	BLOOD SUGAR VALUES AT									FINDINGS AT TERMINATION OF EXPERIMENT			
				Initial	1.5 hours	3 hours	4 hours	5 hours	6 hours	7 hours	8 hours	9 hours	Period	Muscle sugar	Muscle sugar after HCL	Muscle glycogen
	mgm. per kilo	*hour*	*mgm. per kilo*										*hour*	*mgm. per 100 grams*	*mgm. per 100 grams*	*mgm. per 100 grams*
1	0.04	1.5	0.06	87	220	167	107						4	94	149	131
2	0.022	3.0	0.07	98	222	264	132	105	107	101			7	81	141	76
3	0.05	3.0	0.18	100	282	282	196	105					5	67		40
4	0.06	3.0	0.18	117		312	187		122	93	120		8	80		
5	0.09	3.0	0.26	100	308	328							3	161		52
6	0.10	3.0	0.30	98	205	254	142	93	101			105	9	45	171	104
7	0.07	5.0	0.35	118		378		230	116	100	91		8	61		
8	0.07	5.0	0.36	95				296		121			7	66		
9	0.03	6.0	0.18	100	288		380		308				6	98		89
10	0.03	6.0	0.20	118		298		250	200	131	130		8	64	248	87
11	0.035	6.0	0.25	77		154	157		120				6	78	240	92
12	0.04 0.10	5.0 1.0	0.29	85	286	250	250	242	258				6	76	209	54
13	0.04	7.0	0.30	80		222				145			7	56		
14	0.025 0.056	4.0 3.0	0.25	91	145		129		156	240		124	9	53	186	41
	Control for expt. 1 and 2 (A litter mate fasted same length of time)													97	167	215
	Control for expt. 3 and 5 (A litter mate fasted same length of time)													107		330
	Control for expt. 4 and 7 (A litter mate fasted same length of time)													91		
	Control for expt. 6 (A litter mate fasted same length of time)													78	176	405
	Control for expt. 8 and 13 (A litter mate fasted same length of time)													86		
	Control for expt. 9 (A litter mate fasted same length of time)													101		220
	Control for expt. 10 and 11 (A litter mate fasted same length of time)													111	304	429
	Control for expt. 12 (A litter mate fasted same length of time)													97	256	111
	Control for expt. 14 (A litter mate fasted same length of time)													109	214	320

fore, be directly attributed to depletion of the glycogen reserves. The low glycogen values obtained in our series are in perfect agreement with the studies of Sahyun and Luck (1929), who found a rapid fall in muscle glycogen immediately after the injection of 0.5 mgm. adrenalin per kilo and persisting as late as the forty-second hour.

Hydrolyzable sugar. In five of the present series of experiments the muscle extracts were subjected to dilute HCl hydrolysis. The concentration of HCl was adjusted to 1.5 per cent. The time of heating varied from 1.5 to 3.0 hours. In some series the hydrolysis was performed before the removal of protein and creatinine, in others after the removal of these constituents. The reducing power of the hydrolysate is reported as glucose. The values cannot be regarded as absolute, but as control litter mates were used in each series, a basis of comparison is made possible. Palmer (1917) determined the reducing power of muscle extracts after the administration of glucose and several hours after the subcutaneous injection of adrenalin. He concluded that "although the administration of sugar both in normal and diabetic animals increases the amount of dextrose found after hydrolysis, when this amount is compared with the sugar present as dextrose the percentage increase is about the same with or without sugar administration." In all but one of our series the glucose value after hydrolysis was 10 to 20 per cent lower for the animals which received adrenalin. This difference represented very roughly the difference between the muscle sugar values of the control and adrenalinized animals before hydrolysis. In one series the blood sugar was 258 at the time of killing. The muscle sugar was lower than the control muscle sugar. The glucose value after hydrolysis was 20 per cent lower than the control glucose value after hydrolysis. The results would indicate that under conditions in which blood sugar, muscle glycogen, and muscle glucose are affected by adrenalin the polysaccharide sugar of the muscle is not affected.

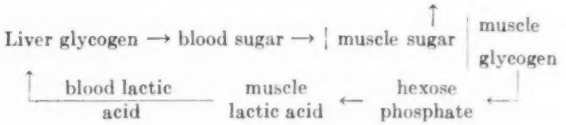
Discussion. The sequence of events relating to the effect of adrenalin upon blood sugar, blood lactic acid, muscle glycogen, muscle lactic acid, and liver glycogen are generally conceded to be well established. The present study aims to correlate the rôles of the muscle glucose and muscle polysaccharide. In the normal resting animals the relation may be expressed schematically.

Liver glycogen → blood sugar → muscle sugar

→ muscle glycogen.

During the period of adrenalin activity (hyperglycemia) there is a decreased utilization of glucose by the muscle. During this period the muscle sugar, though higher than normal, is considerably lower than the blood sugar (at the third hour it may be normal). The reduced muscle sugar concentration

at this stage indicates a vaso-constriction in the muscles even with doses of adrenalin which will not affect the blood pressure. At this stage not only less sugar is utilized by the muscle but actually less sugar reaches the muscle.



It is questionable whether any muscle glycogen is synthesized during this stage. It is, however, possible that the rate of breakdown of glycogen exceeds the rate of synthesis. Falling blood lactic acid values observed in two experiments at the third hour of the injection when the blood sugar values were at a maximum would indicate that the rate of synthesis of glycogen was materially decreased. In these experiments the muscle glycogens were depleted to minimum values of 40 and 52. In the experiments of Cori and Cori (1930), the blood sugar and lactic acid run parallel at the time when the muscle glycogen is rapidly disappearing (the first hour). If the glucose → glycogen → lactic acid reaction continued at the same rate during the following period, the blood lactic acid values would not be expected to fall.[1] It is during this period that Chaikoff and Weber (1928) demonstrated the conversion of fat to carbohydrate, and it would appear that with the depletion of muscle glycogen and the decreased utilization of blood glucose by the muscle, muscle metabolism is forced to concentrate in another direction. In rabbits deglycogenated by synthalin and in which the liver was unable to store glycogen, Bischoff and Long, (1930), were able to

[1] There is an alternative explanation of the fall in blood lactic acid at the third hour on the basis of an increase in the activity of the liver in converting lactic acid to glycogen. Sahyun and Luck showed rather conclusively that during the action of subcutaneously injected adrenalin, the liver glycogen which had been rapidly reduced the first hour accumulated the following hours. We found that with rabbits in which the livers had been poisoned by synthalin, the peak of the lactic acid curve was the fourth hour, following adrenalin given subcutaneously. These observations would tend to place the burden of reducing the blood lactic acid the third hour upon the liver, and would not require a decrease in the glucose → glycogen → lactic acid reaction. However, in our synthalin series the rise in blood lactic acid the fourth hour was observed in animals in which the muscle glycogen values of control and adrenalin dosed animals were nil. Since the liver was definitely proven to be incapable of synthesizing glycogen from lactic acid under the experimental conditions, two possible explanations were given: 1, the muscle utilized less lactic acid, or 2, the muscle formed lactic acid from a source other than glycogen. An explanation based on the reaction glucose → glycogen → lactic acid was here highly improbable, since it necessitated not merely that the reaction continue at the same rate, but also that the rate be increased. Since the glycogen was already depleted and since the utilization of glucose by the animal was decreased 10 to 30 per cent, the reaction could not be accelerated.

estimate quantitatively the utilization of exogenous glucose by the animal with and without the simultaneous action of adrenalin. A 10 per cent and a 30 per cent decrease in utilization under the control animal were demonstrated with adrenalin. Considerable quantities of blood sugar are consequently still used by the muscle during adrenalin activity.

Immediately following the adrenalin activity the muscle sugar falls below normal providing a sufficiently large dose of adrenalin has previously acted (at a physiological rate over a sufficiently long period of time). Since the fall in muscle sugar did not occur when a small amount of adrenalin was given (expt. 2), even though the muscle glycogen depletion was as great, it is quite improbable that the low muscle sugar observed under certain conditions is due to a rapid rate of conversion of glucose to glycogen in the recovery period. The argument is strengthened by the observation that a low muscle sugar value (45) was observed with a relatively high glycogen value (104) at a period when the muscle glycogen is known not to change.[2] The possibility remains that during the adrenalin activity a reserve of unknown chemical constitution is depleted and that during the recovery period the muscle sugar is rapidly utilized, either to build up this store or to take its place. The fall in muscle sugar does not occur unless four or more times the minimum hyperglycemia dose is given. The dose to be effective must be intensive or prolonged so that the store may be depleted. It is quite obvious that under such a condition the muscle glycogen could not be built up, which is in agreement with experimental observation. The experiments show rather conclusively that the unknown reserve could not be the muscle maltose (hydrolyzable non-glycogen carbohydrate).

The question arises as to whether the depletion of the muscle sugar is a physiological process. A physiological rate of adrenalin over a short period of time will not produce the effect, though a pharmaceutical dose will. A physiological rate of injection over a long period of time is effective, however. Does the body ever produce adrenalin continuously over a five hour period, insufficient to raise the blood pressure but sufficient to produce a hyperglycemia?

SUMMARY

1. The fall in muscle sugar below the resting level is observed during the recovery period following a subcutaneous or continuous intravenous dose of adrenalin.

[2] The danger of applying the principles of chemical kinetics to an environment as complex as the muscle is realized, especially when the fate of the reaction products, and the intermediate products are not known. The method appears nevertheless to be the only one available to establish the chemical mechanism of the "normal physiological process." In the present instance the quantitative determination of the muscle lactic acid, hexose phosphate and lipins at time intervals will undoubtedly hasten the ultimate solution of the problem.

2. The effective subcutaneous and continuous intravenous muscle sugar lowering doses of adrenalin are the same.

3. The minimum muscle sugar lowering dose of adrenalin is greater than the minimum hyperglycemic dose. A physiological rate of injection over a long period of time reduces the muscle sugar below normal.

4. When the muscle sugar was observed to be low following the action of adrenalin, the muscle glycogen was also low, but normal or high muscle sugar values were found with low muscle glycogen values.

5. Under conditions in which blood sugar, muscle sugar, and muscle glycogen were affected by adrenalin, the polysaccharide sugar of the muscle was unaffected.

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STUDIES OF SINGLE MUSCLE FIBRES

II. A Further Analysis of the Grading Mechanism

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In a previous paper (Gelfan, 1930) it was demonstrated, by applying microelectrodes to a naturally isolated striated muscle fibre of the frog's retrolingual membrane, that graded electrical stimuli evoked graded contractions. Recent work on the same tissue (Fischl and Kahn, 1928; Pratt, 1930; Hintner, 1930) has shown similar gradations under various conditions, failure of gradation under others. Gradation is, according to Hintner, a sign of injury by cutting, but in earlier and present experiments we have cut wide of the fibres studied and still observe it. A seriously injured fibre rarely can be made to contract at all. A more important source of discrepancy is to be seen in the type of electrodes used. Unless microelectrodes are applied closely to the fibre it is very dificult to obtain the submaximal responses. We have often observed that a fibre giving extensive gradation of response will revert to an all-or-nothing behavior if one of the electrodes touching it is withdrawn a little way. Similarly, when larger metal core electrodes are used in place of the fine fluid ones, gradation is obtained with much greater difficulty. (See also Pratt.)

This relationship of local application to local response is of some significance for the thesis we wish to develop here; one which tends to reconcile conflicting findings and to more accurately define the nature of the all-or-nothing phenomenon.

Assume the existence in the muscle fibre of a series of transverse units arranged along the fibre length—possibly the individual sarcomeres. These units possess contractility and are independent of one another; that is, the contraction of one does not, per se, lead to the contraction of adjacent ones. That such an independence of response, despite protoplasmic continuity, is possible follows from the present findings as well as observations of others on heart. Thus, with weak stimuli, the entire fibre does not twitch feebly, but only a bit of the fibre adjacent to the electrodes twitches. With stronger stimuli more distal portions enter in and so on.

[1] We are indebted to Eli Lilly & Co. for the use of their quarters and facilities at Woods Hole, kindly put at our disposal by Dr. G. H. A. Clowes.

The contraction of each such unit might be all or nothing or graded. The present evidence suggests but does not prove the latter. Normally, the individual contractile units are activated by an excitation transmitted along a muscle fibre in the same manner as along nerve. This is probably a membrane change propagated along the myolemma and obeys the all-or-nothing law, as, so far as most of the evidence indicates, transmission in nerve does. Since this stimulus reaches all the contractile units and is always of maximal intensity, the contractile response as usually observed is also maximal, or zero. This is equally true whether the transmitted wave of excitation in muscle is evoked by a nerve impulse or a diffuse electric current. Since, in most experiments previously reported, the point of observation of the muscle response has been at considerable distance from the point of stimulation, no response was observed unless the transmitted impulse (in the membrane) was initiated and then, of course, the response was maximal (Pratt, 1930).

In Gelfan's experiments, the stimuli were applied to the very region studied for response. It is possible that weak currents, as used here, may directly excite the local contractile mechanism without being strong enough to initiate the membrane response and conducted excitation. A slightly stronger current spreads to adjacent contractile units and gives a stronger twitch and so on, increasing contraction accompanying increased stimulus until finally a propagated disturbance is evoked which sweeps over the muscle fibre in an all-or-nothing fashion.

The effect of puncturing the muscle cell membrane with an electrode is in accord with this view.[2] Here the inner contractile mechanism cannot be reached without first injuring the transmitting membrane, so that the generalized response of the whole fibre is to be expected. A tetanus of the whole fibre begins as the membrane is perforated and ions can flow across

[2] Piercing the sarcolemma with a microneedle always sends the fibre into a tetanus. The duration of the tetanus varies even in the same fibre with different punctures. It may last from some fraction of a second to one or two seconds. Similarly does the relaxation vary. The contraction upon mechanical stimulation is instantaneous, whereas the relaxation is comparatively slow, as if successive activations evoke progressively smaller responses. The extent of injury and recovery depends on the method by which the fibre is punctured. Pulling and stretching of the fibre, as a rule, does not cause it to contract, but a needle puncture will always evoke a response as long as the fibre remains excitable. If the needle is kept inside the fibre after the puncture is made, the tetanus lasts longer and the fibre will lose its irritability sooner. With the needle still inside, the fibre will eventually relax. Upon withdrawal of the needle another response is evoked because of the renewed injury to the healed surface of the membrane. The fibre relaxes sooner from contraction and is least injured when it is punctured by sharp and quick thrusts of a very fine needle. This reaction may be repeated about ten times in a single fibre before irritability is lost, each succeeding puncture being made as soon as the fibre relaxes from its previous contractions. This behavior is strikingly similar to that of Lillie's iron wire model.

the break, stops as the injury seals around the needle and reoccurs still more prolongedly when the entire hole is exposed by withdrawal of the needle and sealing over is slow. Here, surely, the general conducting mechanism is involved and the response appears to be all-or-nothing.

Lillie (1929) has considered the relation of electrode area to activation of a passive iron wire; and points out that with a sufficiently small activated surface area, as produced by a fine scratch of a passive wire, an explosive transmitted response is not obtained, although a heavier scratch is effective in eliciting this response. The small active area produced in the first case may spread slightly but decrements to zero.

In the experiments on muscle, we have also observed very occasionally that scratching a fibre led to feeble localized twitches, in contradistinction to the all-or-nothing response on piercing the membrane. It is not impossible that the local contractions obtained with finely applied electrical currents have associated with them ripples of decremental transmission arising from the cathodally activated area and rapidly fading out. This would be in a sense analogous to a "transitional decrement" seen in nerve responding in all-or-nothing fashion. Such a decrementing transmission is not in any theoretical conflict with existing views of the nature of nervous transmission.

On the interpretation of stimulating current spread, which seems preferable for this case, the all-or-nothing law becomes more strictly defined as a property of *transmission* in nerve and muscle alike. Contraction, normally evoked by a maximal transmitted excitation, also usually appears to be all or none. But contraction may be brought about directly by stimuli that are too feeble to call forth generalized conduction, and the pure contraction response thus isolated does vary in magnitude—in part by change in the number of intracellular units involved, in part, probably, by graded response of each unit. That muscle may contract locally on stimulation when it can not transmit the excitation to all parts of the muscle has often been shown (myoidema, chloroform narcosis, Tiegel contracture, etc.), but the reverse condition has not been obtained.

The crux of this interpretation is in the assumption that contractile units may be directly excited by currents unable to initiate the conducted response, and that such units even at considerable distances from the electrodes may still respond to the stimulating current itself before any true transmission is called forth. If this be true, gradual increase of the stimulus should lead to responses progressively further along the fibre up to a certain point only. When finally the threshold for a transmitted response is reached the entire fibre should suddenly go into maximal contraction.

We have tested these relations on the retrolingual membrane using various types of microelectrodes. The needles were placed under usual magnification and then the nose of the 16 mm. objective removed and a

demonstration ocular introduced permitting observation by two persons simultaneously.

With the low magnification used, a 2.5 mm. length of a single fibre (about half the total maximal length) could be seen. The electrodes were placed close together near one edge of the field and one observer directed his attention to muscle twitches in their vicinity. The other observer watched the distal portions of the same fibre. The extent of the contraction could be well determined and the degree fairly well. Responses to single shocks and tetani of gradually varied strength were studied. With coarse or poorly applied electrodes only all-or-nothing responses were discernible at this magnification. With fine well applied electrodes the usual graded responses were obtained. As stimulus strength increased, the muscle responded for progressively greater distances from the electrode. But even under the best conditions for gradation this spread was not more

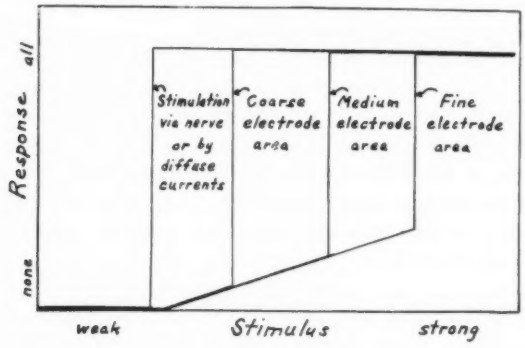

Fig. 1

than about a millimeter; and the more distal part of the responding region was obviously in submaximal contraction. Without exception, the slightest further increase in stimulus evoked a typical maximal twitch of the entire fibre. Hintner (1930) observed a similar effect in some of his experiments, when the spread of contraction had reached only 300μ. As the current becomes less accurately applied to a tiny area of the muscle membrane, the all-or-nothing response appears earlier and the local graded response is cut off sooner, the limit being a completely all-or-nothing contraction (fig. 1).

The results described, though in full harmony with the above interpretation, do not invalidate other possible ones. Decremental conduction breaking over into decrementless conduction would produce a similar effect. We do not see any advantage, however, in considering such an explanation. The sudden change from decremental to decrementless conduction would

still remain a question. It may be urged that a local current spread of a millimeter without stimulation of the conducting mechanism is unreasonable. That the spread is in fact of this order at the time the transmitted response of the whole fibre appears, is shown by the behavior of adjacent ones. We have often noticed a fibre about half a millimeter or more from the stimulated one contract maximally (never graded) at the same time as the one being studied gave a maximal response or when the stimulus was but slightly further increased.

It is rather more difficult to understand the failure of the applied currents to evoke transmission than to understand their ability to call forth responses of the contractile mechanism at some distance. The effect of finer localization of the electrode areas to smaller and smaller regions of the myolemma is to lessen the ease of eliciting the conducted response but not the contractile one. Of course close approximation of the electrodes to the fibre increases the current flow through its interior where, presumably, it reaches the contractile regions more or less diffusely. The current density is, however, greatest right at the membrane itself. Its failure to initiate a propagated membrane change may depend on the very small area of membrane activated, and the close contact of the wall of the electrode with the membrane—preventing the establishment of local circuits between the interior and outer surface of the membrane. Ultimately, of course, with increasing current, enough spread occurs even from the finest electrode to activate the adjacent membrane and the transmitted all-or-nothing response follows.

SUMMARY

The response of a single muscle fibre to induction shocks applied by microelectrodes has been studied. As stimulus strength is increased the length of fibre responding and the size of the response similarly increases. This parallelism continues longest when the electrodes are finest and most closely applied to the fibre but even then, after the contraction has spread a distance of about 1 mm., an all-or-nothing response of the entire fibre breaks through with further increase of stimulus intensity. This is in accord with an interpretation of the graded responses that more accurately defines the all-or-nothing phenomenon and which is developed in the text.

We are indebted to Prof. R. S. Lillie for valuable criticism of this paper.

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BÊTA-TETRA-HYDRO-NAPHTYLAMINE HYPERTHERMIA AND FAT METABOLISM

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Since R. Stern in 1889 (1) first described the hyperthermic properties of Bêta-Tetra-Hydro-Naphtylamine, several authors have studied the pharmacological effects of this drug. For most of them the hyperthermia produced by injection of B.T.H.N.[2] has a central origin (Hashimoto, 2; Mutch and Pembrey, 3; Jonescu, 4; Ott and Scott, 5. Isenschmid, 6) however, and recently also Takahashi Wataru (7) still have observed a hyperthermic reaction after injection of B.T.H.N. in animals who were previously deprived of their thermoregulator centers.

The influence of B.T.H.N. on metabolism, and especially on carbohydrate metabolism has been studied also; Schut (8) has pointed out that in rabbits and guinea pigs a diminution of liver glycogen occurs under influence of B.T.H.N.; this liver glycogen, thus liberated, is the origin of the hyperglycemia; the degree of hyperthermia reached by the rabbits injected with B.T.H.N. would also, following Schut, depend on the amount of glycogen stored in the liver. These views were in opposition with the opinion of Ott and Scott (5) who in previous experiments still observed hyperthermia in animals who were deprived of their glycogen by administration of epinephrin and strychnin.

Since the endocrine glands play an important rôle in the metabolism, one of us was interested, in collaboration with C. Heymans (9) in examining the influence of several endocrine glands on the production of B.T.H.N. hyperthermia. We found that either after thyroidectomy, or after adrenalectomy or after denervation of both adrenals, one could still observe B.T.H.N. hyperthermia. In opposition to the observations of Borchardt (10) on cats, we also obtained B.T.H.N. hyperthermia in dogs with adrenals and thyroids removed. Naphtylamine hyperthermia also occurs in dogs without pancreas (11) and in dogs with denervated liver (12). The only case in our experiments where dogs did not present any increase in temperature after injection of B.T.H.N. was when they had their liver removed (11): this was observed although the animals were in good general

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[2] B.T.H.N.: Bêta-Tetra-Hydro-Naphtylamine.

conditions. The absence of a hyperthermic reaction in those animals did not seem to be due to the absence of liberation of glucose by the liver: injections of amounts of glucose corresponding nearly to the quantities that could be liberated by the liver did not reëstablish the hyperthermic reactions of B.T.H.N.

The question arises as to what materials the animal is using up for the increase of metabolism observed during B.T.H.N. hyperthermia. One could admit that in normal conditions the increase of metabolism that is necessary for the production of hyperthermia is due to the oxidation of liver glycogen. Ott and Scott (5) however had observed that even when an animal is deprived of his glycogen, there still could be produced B.T.H.N. hyperthermia. The opinion of those authors was that in such a case, the hyperthermia was due to a using up of the proteid. Schut (8), however, thought that in Scott's experiments there was still enough glycogen left to explain the rise of temperature after injection of B.T.H.N.

We wanted to see in a series of experiments if there was any influence of B.T.H.N. hyperthermia on fat metabolism. A convenient way to examine this question was to determine the fat content of an organ that plays a very important rôle in fat metabolism, namely, the liver.

Our experiments were made on dogs; under very light ether anesthesia the abdominal wall was incised, the extremity of a liver lobe was tied off with a piece of rubber and the part of the liver that was outside of the ligature was taken for fat, and occasionally also for glycogen determination. The abdominal wall was then immediately closed and the animal left for a while till he was apparently restored. A hyperthermic dose of B.T.H.N. was then injected and the rectal temperature taken regularly during the experiment. When a high degree of hyperthermia was attained, a second sample of liver was taken in the same way as described above and a determination of fat and occasionally of glycogen was made.

The determinations of fat were made in the following way: a piece of liver was weighed on a filter paper and put into an oven till it was completely dry. The fatty substances were then extracted with ether in a Soxhlet apparatus. Afterwards, the sample was dried again and the difference between the weight before and after extraction gave us the amount of fat contained in the sample of liver.

The glycogen determinations were made by the method of Pflüger.

In some experiments, determinations of blood sugar were also made by the method of Shaeffer-Hartmann.

In order to diminish the liver glycogen and also to increase the fat content of the liver and to obtain in this way more typical results, if any difference in the fat content of the liver could be observed, some of our dogs were fed during several days before the experiment only with fat. Some other dogs were fed on a normal diet.

The following experiment shows the influence of B.T.H.N. hyperthermia on the fat content of the liver in a dog who was fed with fat only for several days.

Dog 8.5 kgm., fed with fat only for 4 days.

13.50′ Sample of liver for fat determination: 15.7 grams.
Water content of liver: 74.1 per cent.
Fat content of liver: 13.7 per cent of dry weight.
14.25′ Blood sample at saphenous vein: blood sugar: 1.06 per cent.
14.29′ T. 40°6.
14.30′ Subcutaneous injection of 25.5 cc. B. T. H. N. hydrochl. 1 per cent.
14.55′ T. 41°1.
15.10′ T. 42°.
15.23′ Blood sample taken at saphenous vein: blood sugar: 0.88 per thousand.
15.26′ T. 42°5.
15.40′ T. 42°6.
17.30′ T. 42°6.
17.34′ Blood sample taken at the heart: blood sugar: 0.88 per thousand.
17.37′ Sample of liver for fat determination: 13.973 grams.
Water content of liver: 71.8 per cent.
Fat content of liver: 13.4 per cent of dry weight.

TABLE 1

DOGS	TEMPERATURE		FAT CONTENT OF LIVER (IN DRY WEIGHT)		WATER CONTENT OF LIVER	
	Initial	Maxima	Before	After	Before	After
kgm.			*per cent*	*per cent*	*per cent*	*per cent*
10.5	40° 2	43° 6	8.9	10.4	69.5	73.9
6.0	38° 4	42° 2	18.7	18.47	82.85	83.69
11.6	40° 3	41° 6	28.57	22.74	72.1	70.7

In this experiment, after an increase of temperature of 2°, there is no marked difference between the fat content of the liver before and after the hyperthermia (13.7 and 13.4 per cent). The usual hyperglycemia that one observes after injection of B.T.H.N. did not occur in this experiment: the reason for it is probably that the dog in this experiment did have only small amounts of glycogen stored in the liver. Another fact is to be observed here also: there is no marked difference between the water content before and after the hyperthermia (74.1 and 71.8 per cent).

The next experiment also shows the influence of B.T.H.N. hyperthermia on the fat content of the liver in a dog fed with fat only several days.

Dog 5 kgm.—since 10 days fed only with fat.

11.00′ Sample of liver for fat determination: 10.7 grams.
Water content of liver: 84.5 per cent.
Fat content of liver: 25.2 per cent of dry weight.
Glycogen in liver: 0.674 per cent.

Dog 5 kgm.

11.38′ T. 38°8.
11.44′ Subcutaneous injection of 15 cc. B.T.H.N. hydrochl. (1 per cent).
13.13′ T. 41°35. Polypnea—Dilatation of the pupils.
13.54′ T. 40°75.
15.5′ T. 40°8.
15.10′ Sample of liver for fat determination: 6.8 grams.
Water content of liver: 67.6 per cent.
Fat content of liver: 25.5 per cent of dry weight.
Glycogen in liver: 0 per cent.

In this experiment the marked increase of temperature provoked by injection of B.T.H.N. is not accompanied by any change in the fat content of the liver (25.2 per cent before, 25.5 per cent after the hyperthermia). The water content of the liver is slightly decreased (84.5 to 67.6 per cent). In this experiment the dog had only a very small amount of glycogen in his liver before the experiment (0.674 per cent) and it had completely disappeared after the hyperthermic reaction.

Table 1 represents some other experiments made under the same conditions.

We see in the 3 experiments mentioned in this table, that in two cases (dogs 10.5 kgm. and 6 kgm.) there is an increase of fat content of the liver accompanied with a slight increase in the water content of this organ; in the third experiment a decrease of fat content of the liver goes together with a slight decrease in the water content.

Some other experiments were made on dogs on a normal diet.

These are two examples of those experiments.

Dog 11 kgm.

2.20′ Sample of liver: 8.4 grams.
Water content of liver: 72 per cent.
Fat content of liver: 2.4 per cent of dry weight.
Liver glycogen: 8.13 per cent.
3.45′ Blood sample taken at the saphenous vein. Blood sugar: 0.8 per thousand.
3.46′ T° 39°.
3.49′ Subcutaneous injection of 0.33 gram B.T.H.N. (carb.).
4.39′ T° 39°1.
5.41′ T° 41°2.
6.10′ T° 41°2.
6.12′ Blood sample taken at the saphenous vein. Blood sugar: 1.9 per thousand.
7.13′ T° 42°2.
10.25′ T° 39°.
10.42′ Sample of liver: 11.5 grams.
Water content of liver: 73.2 per cent.
Fat content of liver: 9.2 per cent of dry weight.
Liver glycogen: 0.800 per cent.

In this experiment a marked increase of temperature (3.2) is accompanied by a considerable decrease of liver glycogen (8.13 to 0.800 per cent), a

hyperglycemia (0.8 to 1.9 per cent) and an increase in fat content of the liver (2.4 to 9.2 per cent). The water content of the liver does not undergo any marked change.

Dog 12 kgm.

11.00′ Sample of liver for fat determination: 6.7 grams.
Water content of liver: 66.2 per cent.
Fat content of liver: 6.3 per cent of dry weight.
13.32′ T. 38°.
13.34′ Subcutaneous injection of 36 cc. B. T. H. N. hydrochl. 1 per cent.
15.53′ T. 40°8.
16.35′ T. 40°5.
16.40′ Sample of liver for fat determination: 8.4 grams.
Water content of liver: 70.9 per cent.
Fat content of liver: 7.5 per cent of dry weight.

In this experiment, after a hyperthermia of ±2°, only a very slight change in the fat content of the liver is to be observed. The water content of liver as in the other experiments does not undergo any considerable change.

This series of experiments shows that there is no constant relation between the fat content of the liver and the hyperthermia induced by injection of B.T.H.N. in the dog. In some cases the fat content is increased, in other cases it is decreased and in some experiments it does not change. The exchanges of fat between the liver and the tissues thus seem to be independent of the hyperthermic reaction of the animal. Consequently the animals who are poor in glycogen do not use up the stored liver fat to increase their caloric production during the B.T.H.N. hyperthermia. It is probable that the increase of metabolism in this condition is due to using up of the protein after the carbohydrates have been depleted.

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THE INDUCTION OF THE PSEUDO-PREGNANCY VAGINAL REACTION IN SPAYED MICE BY THE INJECTION OF HUMAN BLOOD

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The recognition of cyclic changes in the vaginal mucosa of some laboratory animals (Stockard and Papanicolaou; Evans and Long; Allen) has given a tremendous impetus to the study of female sex hormones since it has made possible the development of certain biologic tests for these substances. The main interest has centered on the induction of estrual changes ("cornification" of the vaginal epithelium) in spayed mice as a test for estrin, but the recent work of Wiesner and his co-workers (1929, 1930) points to the importance of an altogether different histologic transformation of the vaginal mucosa ("mucification") which occurs during the "reproductive" as contrasted to the "estrual" phase of the sex cycle in mice. This change is similar to the "pseudo-pregnancy" induced in rats by the mechanical inhibition of estrus (Evans and Long, 1921) and has been produced by the injection of various extracts of the corpus luteum. The present study is an analysis of such changes induced in the vaginae of adult spayed mice by the administration of blood serum from patients in order to determine if this method offers an additional biologic test which may prove of clinical assistance.

The test as developed in the Stanford Gynecologic Laboratory consists in the injection of clear blood serum into a medium-sized spayed mouse.[1] The injections are carried out two or three times daily over a period of three to four days and until the animal has received a total of from 6.0 to 10.0 cc. of the serum. The mouse is sacrificed on the fourth or fifth day and the vagina is then carefully dissected out, fixed in formalin, and mounted in paraffin. A number of sections are taken at different levels and stained with eosin and hematoxylin. Since the vaginal smear found accompanying mucification of the vagina is almost identical to that occurring normally

[1] Although Wiesner used immature animals in his work it seemed inadvisable to employ them in these experiments since many of the specimens of blood (for example, during pregnancy and following castration) contained anterior lobe hormone. This would have resulted in a stimulation of the immature ovary with a secondary change in the vaginal mucosa, thus masking any direct effect on this tissue.

during diestrus it has been impossible to use this method and microscopic sections were thus necessary to establish the diagnosis.

An analysis of the histologic findings in the vaginae of the mice now showed that they may be grouped into three main categories, namely, 1, negative findings and control animals; 2, cornification (fig. 1a) due to the predominant effect of estrin, and 3, mucification which has been subdivided into three groups representing quantitative effects.

Negative results and controls. The animals failing to respond to the injections and 9 non-injected mice examined from 10 to 20 days after castration, show a vaginal mucosa in a state of atrophy. The epithelium is composed of two rows of cells with a surface layer of small cuboidal cells with clear

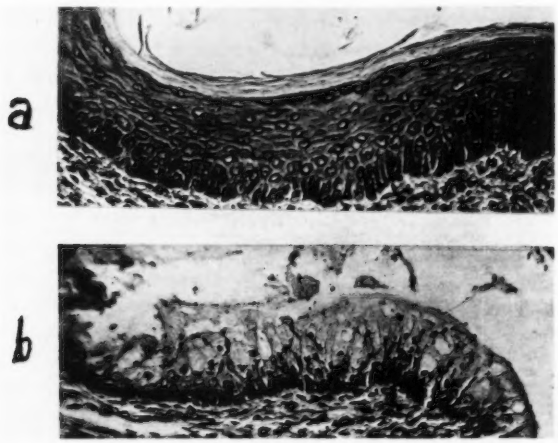

Fig. 1. Photomicrographs of same magnification showing *a* cornification, and *b* mucification, of the vaginal mucosa of mice.

protoplasm. There is almost no leucocytic reaction, and only an occasional desquamated cell is seen lying free in the lumen of the vagina.

Mucification +. This reaction shows that the mucosa has responded but mildly to the stimulus, and is comparable to that described by Wiesner and Crew (1930) as induced in the vaginae of immature mice by an "insufficient amount of kyogenic extract" of the anterior lobe. The cells have proliferated so that there may be from three to five layers and a considerable number at the surface are of a larger size, cuboidal in shape, and show a clear protoplasm. Certain groups are also found which are identical to those seen with + + mucification but this change is not general. There is, however, an intense leucocytic reaction, and in the lumen of the vagina there is a collection of mucus with numerous polymorphonuclear leucocytes and desquamated nucleated epithelial cells.

Mucification ++. In this case the change has progressed further, and although there is a similar leucocytic invasion and collection of mucus, a much more general transformation is seen in the mucosa. At the surface are now found cylindrical cells, well outlined and with clear protoplasm, and in many areas these are still more developed and correspond to the type seen with +++ mucification. A very similar picture is to be found in mice during the period of lactation.

Mucification +++. The third category represents the characteristic "pseudo-pregnancy" change and corresponds to the picture seen in figure 1b. The surface of the mucosa shows tall, stratified cylindrical cells filled with mucus and resting on a basal layer of small deeply-staining cells. A large amount of mucus with leucocytes and cast-off nucleated epithelial cells is also present in the lumen of the vagina.

The test has now been used to examine the blood of over 70 patients. The examination of 8 women with pregnancies of from 6 to 16 weeks' duration gave +++ mucification 6 times, and + and ++ each once. In one

TABLE 1

Findings during normal menstrual cycle (25 cases)

	1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK
Mucification +++	0	1	1	1
Mucification ++	0	3	3	3
Mucification +	1	1	0	0
Negative	3	2	4	1
Cornification	0	0	1	0

case of late pregnancy (7 months) cornification occurred, thus showing a preponderance of estrin. In 6 patients examined during the first ten days post-partum +++ mucification was obtained 3 times, ++ once, and the test was negative twice; while six weeks' post-partum + mucification was found in one instance. One very interesting finding was obtained in a patient with an ovarian cyst. The reaction was strongly positive (+++) before excision of the tumor, and the histologic examination later showed this to be a corpus luteum cyst. A second specimen of blood was then examined nine days after operation and proved definitely negative. One patient whose menses were one week overdue gave a strong +++ reaction. Of three other patients with a functional amenorrhea two were negative while the third showed a + mucification.

Twenty-five women were examined at different stages of the menstrual cycle, and the findings are charted in table 1. Although varied results were obtained during the second and third weeks, it is of interest to note that during the premenstrual stage 4 out of 5 patients gave ++ or +++ mucification. On the other hand, 3 out of 4 women during the first week proved negative and the fourth only gave a + reaction.

Twelve women with a complete absence of ovarian function were also examined. Of 3 patients who had had a previous bilateral oöphorectomy one gave a +++ mucification. The blood of 9 women past the climateric was examined and two gave a +++ reaction, one a ++, one a +, while 5 were negative. And finally, blood from 12 men gave a +++ mucification twice, a ++ 5 times, a + once, and only 3 proved negative.

Discussion. There is considerable laboratory evidence showing that the induction of mucification in the vaginae of mice may be a test for "progestin", a hormone of the corpus luteum which is responsible for the pregravid and gravid changes that occur in the pelvic organs (Corner et al). In the first place, it is present in varying degrees in the vagina of the mouse at times when the pelvic organs are known to be under the influence of the corpus luteum, namely, during pregnancy and lactation. Secondly, a corresponding change occurs in rats in the "pseudo-pregnancy" elicited by the mechanical inhibition of estrus (Evans and Long, 1921). Thirdly, in the Aschheim-Zondek test mucification is found when the ovaries of the test animal contain only luteinized structures (Wiesner, 1930; Fluhmann, 1930), and this correlation has also been shown by the work of Wiesner (1930), and Evans and Simpson (1930) with "luteinization" extracts of the placenta or anterior hypophysis. And finally, it has been produced experimentally by the injection of extracts of the corpus luteum in immature mice by Wiesner and Patel (1929), in rats by Hisaw et al (1929), and in castrated mice by Clauberg (1930).

The present study shows that mucification of the vaginal mucosa may be induced in spayed mice by the injection of blood obtained from women in whom active corpora lutea are present, namely, in the latter part of the menstrual cycle, during pregnancy, early in the puerperium, and in one case accompanying a corpus luteum cyst. However, there are three main objections to considering this procedure as a reliable test for progestin in the blood of women.

1. The changes are induced without the preliminary administration of estrin, and this would seem somewhat contradictory to the recent studies of Corner, Hisaw, and others. Although in early experiments estrin was administered and the injection of blood serum begun only when the vaginal smear showed cornified cells, this has been found unnecessary, and in fact negative results were obtained by this method, while smaller amounts of the same specimen produced "pseudo-pregnancy" changes in the vagina of an untreated animal. Wiesner and Crew (1930) also note that this finding is apparently independent of any preceding action of estrin. A possible explanation lies in the fact that the human corpus luteum produces estrin at the same time as progestin, and both may therefore be present in the blood in the proper proportions to stimulate the mucification of the mouse vagina.

2. The finding of a large percentage of positive results from the examination of blood from men casts many doubts on the efficacy of the test. Although the occurrence of estrin, the so-called "female sex hormone", in the blood of men has been reported by numerous investigators (Dohrn, 1927; Hirsch, 1928; Loewe et al, 1928; Frank and Goldberger, 1928; Dingemanse et al, 1930), there is as yet no information regarding the production in the male of a second hormone corresponding to progestin. Since the anterior lobe of the hypophysis from males is capable of inducing "luteinization" in the ovaries of immature mice this is not altogether an impossibility, but the whole question must remain open for the present since it is not even proven that estrin is actually produced in men but may come from some outside source, possibly from certain foods (Frank, 1929).

3. And finally, the induction of the pseudo-pregnancy vaginal reaction by the injection of blood from one woman who had had a previous bilateral oöphorectomy and from four past the menopause, demonstrates fairly conclusively that it cannot be considered as one produced solely by a hormone of the corpus luteum.

CONCLUSIONS

A mucification of the vaginal mucosa of spayed mice has been produced by the injection of blood serum from patients in whom it is known there is a histologic corpus luteum, namely, during pregnancy, early in the puerperium, during the premenstrual stage, and accompanying a corpus luteum cyst. However, since it has also resulted from the injection of blood from men, from one woman following a bilateral oöphorectomy and from four women past the climacteric, it does not seem justifiable to consider it as a reliable test for the presence of progestin in the blood.

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MASSAGE OF THE THYROID GLAND AND RATE OF DENERVATED HEART IN THE DOG

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It has been reported by Cannon and Smith[2] that gentle massage of the thyroid gland produces an increase in the rate of the denervated heart of the cat, attaining sometimes to 33 per cent over the initial rate; the increase observed takes place slowly, requires from 30 to 60 minutes before being at its maximum and passes off progressively. This increase also takes place in the absence of the adrenal glands.

Since there are only a few methods to detect thyroid secretion, it would be interesting if it were possible to use Cannon's method to record changes in this secretion. In order to observe if Cannon's observations could be extended to other animals, on suggestion of Dr. A. J. Carlson, we made the following attempts to reproduce these experiments on dogs.

The dogs used in our experiments were under light barbital anesthesia. Our experiments were made in April and May, and at that period of the year the thyroid gland seems to be poor in iodin. In order to have a good content of iodin in the thyroid, we gave our animals, during periods varying from 5 to 12 days before the experiment, each day, 3 to 5 drops of Lugol solution by mouth.

The experiments performed were of two different types.

In the first type, the dog was anesthetized, the vagi and sympathetics cut low in the neck, and both stellate ganglia removed by the lateral route after cutting off a part of the first rib; the resection of the stellate ganglia, in this way, was possible, in most of the cases, without opening the pleural cavity and without resorting to artificial respiration. The blood pressure was recorded by the mercury manometer. After a control period, gentle massage of each of the thyroid glands was made during about two minutes and the rate of the heart was observed on the blood-pressure tracing during some time after the massage.

In a second type of experiment we used two dogs, dog A weighing about one-third of dog B. Both dogs were anesthetized and the cardiac end of the right carotid artery of A was connected by way of a Payr's cannula

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[2] Cannon and Smith: This Journal, 1922, lx, 476.

to the cephalic end of the left carotid artery of B below the thyroid gland. The right cardiac end of the external jugular vein of A was connected by a Payr's cannula to the cephalic end of the left internal jugular vein of B, below the left thyroid gland. The left internal jugular vein and the left carotid artery of B were then tied off above the thyroid gland. Both thyroid glands were removed in dog A and his heart was denervated as in the first type of experiment. The blood pressure of dog A was recorded by a mercury manometer. In this way, the left thyroid gland of B was perfused by A who was himself thyroidectomized and, dog B being much heavier than dog A, the latter was able to receive rather large amounts of

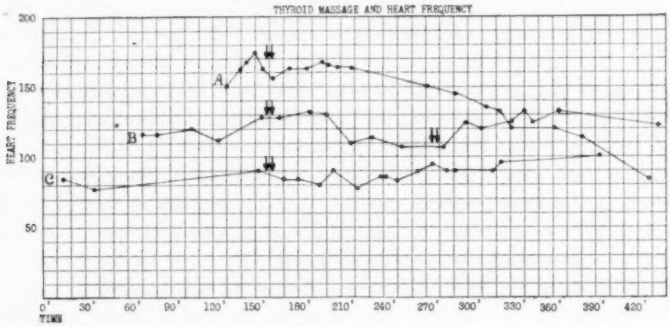

Fig. 1

blood from dog B. We then tried to observe if the massage of the left thyroid gland of B produced an increase in the rate of the heart of A.

The chart presents typical results of our experiments. The tracings represent the frequency of the denervated heart, the massage having been made at points marked by the arrows. Experiments A and C were performed following the first type, experiment B following the second type.

As shown by the chart, we were unable to observe any marked or consistent increase in the rate of the denervated heart after massage of the thyroid gland.

We wish to thank Dr. A. J. Carlson for suggestion of this work, and Dr. H. Necheles for valuable assistance in the experiments.

THE RESPIRATORY QUOTIENT OF RESTING MAMMALIAN MUSCLE AS SHOWN BY THE EVISCERATED DECAPITATED CAT

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The respiratory quotient (R. Q.) observed over a long period under constant conditions indicates the nature of the foodstuff oxidized. Two factors in the respiratory process must be measured; 1, oxygen consumed, and 2, carbon dioxide produced. The measurement of oxygen consumption shows directly the amount consumed for oxidation, for there is no facility for oxygen storage. The carbon dioxide reserves, however, are large, and its elimination is primarily regulated by the necessities of maintenance of the acid-base equilibrium. Blood and alveolar CO_2 tensions may indicate a tendency for CO_2 to be withdrawn from the reserves by over-ventilation or retained by under-ventilation, but quantitative evidence can only be derived from direct examination of an important depôt of reserve CO_2. One important reserve of CO_2 is in the muscles. They contain about two-thirds as much CO_2 as blood per unit of volume, but weigh nearly ten times as much, so that their CO_2 content is over five times greater than that of blood. There is also a further advantage in considering the muscle CO_2 content as a guide to the state of the body CO_2 reserves. The muscles are the site of active metabolism and represent an ultimate source of both metabolic CO_2 and reserve bicarbonate. With the recent development of methods for CO_2 determination in muscle, it is possible to control the measurement of respiratory products by observing whether or not the CO_2 content of muscles remains unchanged during an experiment.

The procedure for determining the R. Q. of mammalian muscle alone involves experimental manipulation which is particularly likely to upset the acid-base equilibrium. For this reason determinations on excised muscle are of doubtful value. Two other methods are available. In the first the abdominal viscera are eliminated and a preparation made which is essentially heart, lungs and muscle. Examination of the gas exchange shows the results of what is principally muscle metabolism. The second method depends upon examination of the arterial and venous blood from an otherwise isolated muscle, with deduction as to the actual gas exchange. Each method presents besides its peculiar technical difficulty that of controlling the acid-base equilibrium.

There are many observations on the R. Q. of muscle as shown by the eviscerated preparation, but the results are not consistent, and in no case has control of the acid-base equilibrium been successfully established. The older observers lacked satisfactory methods, so that their conclusions are either vague or unjustified. The first observations of the recent series were made on the R. Q. of rabbits after tying off the abdominal aorta and vena cava. This leaves an anterior preparation which is chiefly muscular (Porges, 1910). The R. Q. rose to near 1 and remained high longer than was considered possible from the result of over-ventilation. From the rather variable results, Porges concluded that muscles were obligated to exclusive carbohydrate metabolism.[1] Porges and Salomon (1910) likewise concluded that the R. Q. for depancreatized dogs was near one after elimination of the posterior parts and abdominal viscera. Murlin, Edelmann and Kramer (1913) criticized these results and showed that the R. Q. rise followed a fall in arterial blood CO_2 content. When the arterial blood CO_2 content increased, the R. Q. fell. They concluded that the preparation did not demonstrate any change in R. Q. except from alteration of the acid-base equilibrium.

Burn and Dale (1924) used eviscerated cats which had been previously decapitated. The decapitated preparations recovered from anesthesia and were maintained by artificial ventilation so that the gas exchange could be conveniently followed. While the blood sugar was maintained at a high level, the R. Q. remained near one and was not further affected by insulin. Their experiments were, however, incidental to demonstration of the site of insulin action and lacked control of the acid-base equilibrium, so that they should not be quoted as evidence that muscle is obligated to carbohydrate metabolism. The results of Markowitz (1928) showed that after pancreatectomy or total evisceration of dogs the R. Q. was less than one, and that administration of sugar did not raise the R. Q. These results led to the conclusion that extra sugar, at least, was not oxidized by the eviscerated animal.

The conclusion of obligate carbohydrate metabolism in eviscerated cats was criticized by Kilborn (1928). He showed that after evisceration, the O_2 consumption diminished rapidly. Meanwhile, if the artificial ventilation was continued at a rate satisfactory for the decapitated animal, over-ventilation resulted and extra CO_2 was blown off. As evidence he showed the progressive decline in blood CO_2 content, temporary restriction of the rise in R. Q. when CO_2 was added to the inspired air, and the elimination of comparable amounts of extra CO_2 during over-ventilation of decapitated

[1] We regret the omission of reference to Verzár's (Biochem. Zeitschr., 1911, xxxiv, 61) criticism of the conclusions of Porges. Verzár showed that several possible causes of the high R. Q.s were not controlled, and that the results could not be regarded as indicating a change in the type of metabolism.

cats with intact viscera. From these points of evidence he concluded that the eviscerated cat showed no change in R. Q. which could not be attributed to over-ventilation or acidosis, and that muscle is certainly not restricted to the metabolism of carbohydrate. The results of Bornstein (1929) are quite similar. Using eviscerated dogs under chloralose anesthesia, he showed that the rise in R. Q. was attended by diminished alveolar CO_2 tension. As the R. Q. was usually less than 0.9, he concluded that muscle can utilize considerable fat. Administration of glucose raised the R. Q., as if the proportion of carbohydrate oxidized was largely a matter of the amount of sugar in the blood.

If over-ventilation is the cause of the rise in R. Q. after evisceration, quite considerable amounts of CO_2 must be withdrawn from the animal. Blood changes alone had been demonstrated, and they did not account for an appreciable part of the rise in R. Q. Looking for the source of extra CO_2, Ferguson, Irving and Plewes (1929) found that muscle lost a considerable amount, though not enough to account for the whole rise in CO_2. But over-ventilated cats and cats ventilated with CO_2 rich mixtures showed a CO_2 capacity quite adequate to supply the excess calculated as lost by the eviscerated preparation (Irving, Ferguson, and Plewes, 1930).

Most of the evidence indicates that the R. Q. of muscle is not necessarily one. But none of the experiments successfully demonstrate the value of the R. Q. when the acid-base equilibrium is not seriously affected. Allowance for depletion of the CO_2 reserves is not appropriate because they have not all been located. It remains to be determined, then, if the rate of ventilation can be reduced and the acid-base equilibrium regulated both in the blood and muscle reserves.[2] If that is possible, the R. Q. determined will probably be correct. There are also contradictions as to the influence of the abundance of carbohydrate in the blood on the R. Q. of muscle. Lastly, the eviscerated preparations used have shown a disposition toward over-ventilation or acidosis which could not easily be controlled. Will the metabolism of carbohydrate, as facilitated by an abundant supply of insulin, relieve the tendency toward depletion of the tissue CO_2 reserves?

METHODS. The general plan of the experiments was first to decapitate the cats under ether anesthesia and maintain them for one or two hours with artificial respiration until a normal R. Q. had been determined. Blood and muscle samples were removed. The cat was then eviscerated and the rate of ventilation reduced to avoid over-ventilation with the diminished oxygen requirement. Subsequent blood and muscle samples served to control the stability of the acid-base equilibrium. The post-evisceration period lasted for three hours or more.

[2] Kilborn (1928) observed a fall in R. Q. after reducing the rate of ventilation of eviscerated cats, but only did two experiments on account of the resulting anoxemia.

Decapitation and evisceration. Under ether anesthesia the cats were supplied with carotid and tracheal cannulae and decapitated according to Sherrington's method. Evisceration was performed as described by Burn and Dale (1924). Through a long abdominal incision the intestine was tied at the posterior end and cut. The mesenteric and coeliac axis vessels were then ligated and cut, and likewise the portal vein. The whole mass of stomach, intesine, spleen and pancreas was then removed by cutting the esophagus. The liver remained *in situ* but deprived of any active circulation except for the possibility of ebb and flow into the vena cava. In most of the experiments the kidneys were tied off and excised. Venous blood samples were obtained through a cannula in the renal vein from the inferior vena cava.

Artificial respiration was maintained by the convenient Schuster respirometer, made by C. F. Palmer, Ltd., London. The respirometer circulates air through the lungs and a closed circuit in which is situated a counterbalanced spirometer. As the spirometer falls with oxygen consumption, it releases a separate pump which injects O_2 into the system. Each stroke of the O_2 pump is registered by a counter, and the stroke volume can be accurately determined by allowing a measured volume of water to flow from a reservoir placed in the circuit. The volume of air injected into the lungs can be measured by introducing a water manometer into the system. CO_2 absorbers were also placed in the circuit. The absorbent used was ascarite, the fused sodium hydroxide and asbestos preparation of Stetser and Norton (1918), which does not lose water to the stream of dried air. The accuracy of CO_2 determination was checked by decomposition of sodium carbonate. The whole system could be easily checked against possible leaks and for accuracy. With the circulating volume of air used 2 degree changes in room temperature caused no appreciable error. The system gives continuous measurements of O_2 consumption and CO_2 production.

Blood analyses. For determination of total CO_2 and O_2 blood was drawn directly from the carotid artery or from the vena cava through the renal vein into a 1 cc. pipette and analyzed according to the method of Van Slyke and Neill (1924). Samples of about 4 cc. were also drawn and equilibrated with a CO_2 mixture for determination of CO_2 combining power. Two or more combining power determinations were made at long intervals in order to show the extent of change of CO_2 capacity.

Blood sugar determinations were made according to the Shaffer-Hartmann (1920) method, introducing 1 cc. samples of blood directly into water and precipitating protein with tungstic acid.

Muscle CO_2 content. The muscles were carefully dissected out and removed as if for lactic acid determination. They were then frozen in liquid air. Samples of about one gram were broken from the frozen muscle and

analyzed for CO_2 content by the method of Ferguson and Irving (1929). Right and left corresponding muscles examined simultaneously by this method usually differ by less than five per cent of the total amount of CO_2 present. As the determinations were generally made on four pairs of muscles, the trend of the CO_2 content is shown in spite of the variability of the control values.

Muscle lactic acid content. Samples of the muscles used for CO_2 determinations were examined in some cases for lactic acid content by the method of Friedemann and Kendall (1929), following preliminary protein precipitation by the Schenck procedure and the Van Slyke method of eliminating carbohydrate.

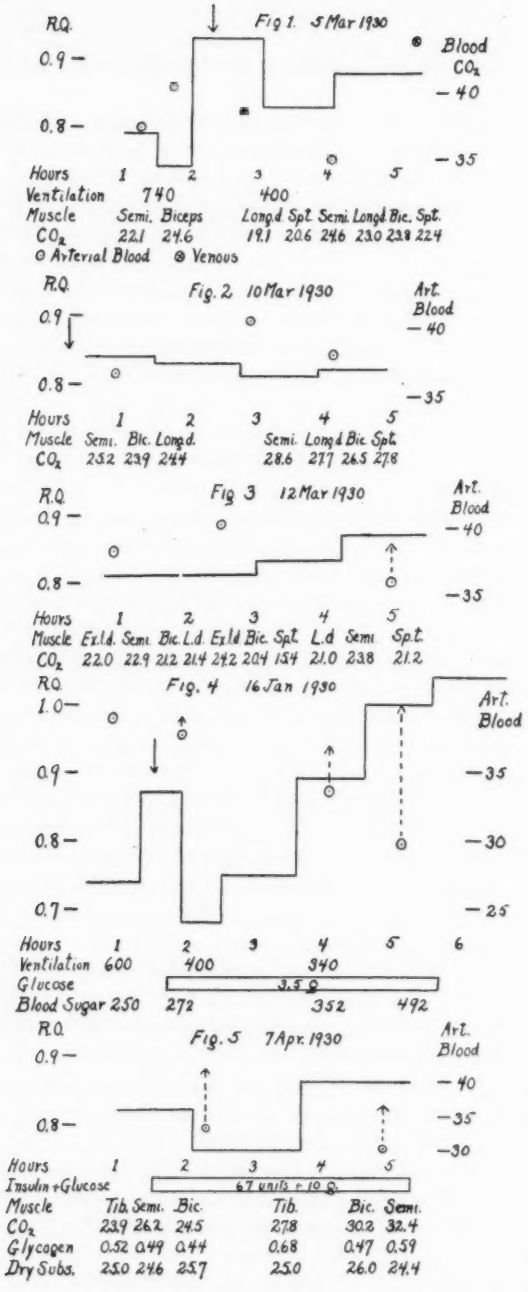
Muscle glycogen. Muscle samples were used in some cases for glycogen determination by the general Pflüger method, estimating the sugar by Bertrand's procedure.

EXPERIMENTAL RESULTS. In table 1 there is shown the protocol of a typical experiment. The cat was decapitated and maintained for 45 minutes at a ventilation rate of 20 pump strokes of 38.5 cc. each per minute. During the next two periods amounting to 75 minutes the R. Q. was steady at 0.77 and 0.76, the O_2 consumption likewise remaining practically constant. During this time the CO_2 content of arterial blood changed only from 47.3 to 49.0 cc. per 100 cc. The blood sugar dropped rapidly as the ether anesthesia and shock of decapitation passed away.

During evisceration the O_2 consumption started to decline rapidly, reaching a fairly steady level at about one-third of the initial rate of consumption. To correspond with the decreased O_2 consumption the pump stroke volume was reduced to 25 cc., the rate remaining constant at 20 per minute. There was a temporary rise in the R. Q. during the stimulation due to evisceration, followed by a drop to about the original level. In the 188 minutes following evisceration the R. Q. had an average value of 0.77, i.e., no change from the value before evisceration.

The correctness of the R. Q. is controlled by determinations of arterial blood and muscle CO_2. The arterial CO_2 content, determined four times after evisceration, showed a progressive loss of CO_2. Taking the blood volume as 5 per cent and the CO_2 loss as 17 cc. per 100 cc., the total amount of CO_2 passing from the reserves in blood alone is $0.05 \times 35 \times 17 = 29.8$ cc., not enough to affect the value of the R. Q. If the CO_2 change in other tissues were proportional to that in blood the effect on the value of the R. Q. would be considerable. But the combining power of the arterial blood falls in a way that is parallel to the CO_2 content, so that the CO_2 tension in the final blood sample is not greatly different from the original CO_2 tension.

The arterial blood CO_2 tension is only one factor in the process of CO_2 elimination. The final criterion is the stability of the tissue CO_2 reserves.


R.Q.
Fig 1. 5 Mar 1930
Blood CO_2
0.9
0.8
40
35
Hours 1 2 3 4 5
Ventilation 740 400
Muscle Semi. Biceps Long'd Spt. Semi. Long'd Bic. Spt.
CO_2 22.1 24.6 19.1 20.6 24.6 23.0 23.8 22.4
⊙ Arterial Blood ⊗ Venous
R.Q.
Fig. 2 10 Mar 1930
Art. Blood
0.9
0.8
40
35
Hours 1 2 3 4 5
Muscle Semi. Bic. Long'd. Semi. Long'd Bic. Spt.
CO_2 25.2 23.9 24.4 28.6 27.7 26.5 27.8
R.Q.
Fig 3 12 Mar 1930
Art. Blood
0.9
0.8
40
35
Hours 1 2 3 4 5
Muscle Ex.l.d. Semi. Bic. L.d. Ex.l.d Bic. Spt. L.d Semi Spt.
CO_2 22.0 22.9 21.2 21.4 24.2 20.4 15.4 21.0 23.8 21.2
R.Q.
Fig. 4 16 Jan 1930
Art. Blood
1.0
0.9
0.8
0.7
35
30
25
Hours 1 2 3 4 5 6
Ventilation 600 400 340
Glucose 3.5 g
Blood Sugar 250 272 352 492
R.Q.
Fig. 5 7 Apr. 1930
Art. Blood
0.9
0.8
40
35
30
Hours 1 2 3 4 5
Insulin + Glucose 67 units + 10 g.
Muscle Tib. Semi. Bic. Tib. Bic. Semi.
CO_2 23.9 26.2 24.5 27.8 30.2 32.4
Glycogen 0.52 0.49 0.44 0.68 0.47 0.59
Dry Subs. 25.0 24.6 25.7 25.0 26.0 24.4

Figs. 1 to 5

These have been controlled by showing that the average muscle CO_2 content remains practically constant. It is apparent that the R. Q. determined is the true one for oxidation.

The results of three similar experiments are summarized in figures 1, 2, and 3. In figure 1 is shown the rise in R. Q. following evisceration, with a subsequent decline to values slightly greater than before evisceration. The CO_2 content of arterial blood was 37.5, and 40.4 before evisceration and fell in $2\frac{1}{2}$ hours to 35.0 volumes per cent. The venous blood CO_2 content increased from 38.6 immediately after evisceration to 43.9 in $2\frac{1}{2}$ hours, the combining power remaining constant. There is not much indication of the general acid-base situation from these figures except that the changes are not great for such a preparation. The muscle CO_2 content determinations are more instructive. During evisceration the R. Q. rose to 0.93, while the muscles lost CO_2. In the periods after evisceration the muscles recovered approximately the amount of CO_2 lost. The sudden rise in R. Q. is attended by loss of muscle CO_2 and is therefore not indicative of increased carbohydrate oxidation. The average R. Q. after the high value of the evisceration period is 0.86, compared with 0.77 before evisceration. The preparation was not so stable in respect to its CO_2 reserves as the others, but it shows clearly how the rise in R. Q. is characteristically attended by loss of muscle CO_2. The experiment also shows that muscle changes are more significant than those shown in the blood.

In the experiment recorded in figure 2 evisceration followed immediately after decapitation and before recovery from anesthetic, in order to reduce shock. The preparation was subsequently examined during $4\frac{1}{2}$ hours while the R. Q. remained between 0.84 and 0.81. The CO_2 content of arterial blood rose slightly from 36.8 to 38.0, while the combining power with 5.5 per cent CO_2 increased from 35.7 to 37.9, indicating only a slight decrease in alveolar CO_2 tension. The muscles showed an average CO_2 gain of 3 per cent, or $0.03 \times 3000 \times 0.5 = 45$ cc. If the 45 cc. of CO_2 retained in the muscles is added to the CO_2 expired, the average R. Q. after evisceration is raised from 0.83 to 0.85. The correction is not significant, but it appears necessary and appropriate in view of the decline of the R. Q. from 0.84 to 0.82 after evisceration.

Figure 3 records an experiment like that shown in figure 2, with R. Q. just below 0.85 during $4\frac{1}{2}$ hours. Except for one discordant value the muscle CO_2 content is unchanged over the period. The arterial blood CO_2 content falls slightly, but when corrected for a small change in combining power, it is evident that the alveolar CO_2 tension is practically constant.

In the last two experiments described the R. Q. after evisceration is just below 0.85 consistently for long periods. There is no evidence that the CO_2 reserves of the animal are altered sufficiently to effect great modification in the R. Q.

In such a preparation as the eviscerated cat the circulatory system has not much ability to compensate for improper ventilation, so that the CO_2 reserves are easily affected. In some experiments it is possible to make reasonable allowances for the effect of changing CO_2 reserves on the R. Q. These allowances are invariably in agreement in a general way, that when the R. Q. after evisceration rises, blood and muscle show the effect of over-ventilation. Taking the ten experiments shown in table 2 that are well controlled and of reasonable duration, the R. Q. after evisceration is usually near 0.8. In seven of these the muscle CO_2 content was determined. Five showed no change, one a small change, and one a change that would raise the corrected R. Q. to 0.91. The latter experiment, as indicated in figure 1, does not show sufficient stability to make its evidence significant against that of the nine others.

The R. Q. before evisceration is usually somewhat lower than after. The three experiments with initial R. Q. less than 0.70 were recovering from previous earlier over-ventilation, and the pre-evisceration R. Q.s may be discarded. The first four experiments then show that the usual R. Q. for the decapitated cat is between 0.75 and 0.80; after evisceration it is usually just above 0.8. There is no suggestion of a considerable alteration in the type of oxidative metabolism after evisceration, and the resting muscle preparation is able to carry on oxidative metabolism with practically the same substances as the intact animal.

Discussion. It had already been shown that the rise in R. Q. after evisceration was usually attended by loss of CO_2 from arterial blood (Murlin, Edelmann, Kramer, 1913; Kilborn, 1928; Bornstein, 1929). But after the profound modification of circulation caused by evisceration, decline in arterial blood CO_2 content or in alveolar CO_2 tension is not sufficient evidence for over-ventilation. Further determination of venous blood CO_2 tension shows the pressure gradient for CO_2 elimination, but it does not indicate the rate of CO_2 loss. Although the difference between arterial and venous CO_2 tension may be large, the amount of CO_2 lost is also determined by the volume flow of blood. Normally the CO_2 loss is well coördinated with the O_2 intake by both vascular and respiratory adjustment, but the regulation of these adjustments is almost absent after decapitation and evisceration. The acid-base equilibrium of the blood is only partial evidence for the existence of unsuitable rates of respiration, and cannot be used to suggest how far the R. Q. is changed from that which indicates the nature of oxidation processes.

When the reserve supplies of CO_2 can be examined, the suitability of the rate of ventilation is at once apparent in their stability or unstability. But since Ferguson, Irving, and Plewes (1929) were not able to locate all of the CO_2 reserves, correction of the R. Q. by the amount of change in muscle CO_2 content is not a sufficient correction. The only condition in which an

R. Q. is really satisfactory is when the CO_2 reserves are unaffected. Muscle is a relatively sensitive indicator of the CO_2 reserve while blood is far too complicated a system to use as an indicator in these experiments. These essential conditions of controlled CO_2 reserve are met in a sufficient number of our experiments to show definitely that the correct R. Q. of the eviscerated preparation, which is principally muscle, is only slightly greater than that of the intact animal and far less than one. Evidently evisceration does not alter the type of foodstuff oxidized, and muscle alone during resting metabolism is not restricted to the oxidation of carbohydrate.

TABLE 1

Eviscerated cat. Weight 3.5 kgm. February 19, 1930

	TIME	VENTILATION RATE	O_2	O_2	CO_2	R.Q.	ARTERIAL BLOOD				SUGAR	MUSCLE CO_2
							CO_2	O_2	Combining power in 5.5 per cent CO_2 in O_2			
									CO_2	O_2		
	minutes	*cc. per minute*	*cc.*	*cc. per minute*	*cc.*		*cc. per 100 cc.*	*cc. per 100 cc.*	*cc. per 100 cc.*	*cc. per 100 cc.*	*mgm. per 100 cc.*	*cc. per 100 grams*
I Decapitated	45	770										
II	31		840	27.1	645	0.77	47.3	11.1			285	31.6 28.1
III	44		1,248	28.3	943	0.76	49.0	10.5	48.5	11.8	150	
IV Evisceration	24	500	441	18.4	399	0.90						
V Post evisceration	86		847	9.9	666	0.79	42.1	12.9	46.0	16.5	112	
VI	53		418	7.9	320	0.77	38.5	12.1			077	
VII	49		355	7.3	257	0.73	37.0	11.1			042	31.4 32.4
							32.4	11.2	28.0	16.9	012	

Himwich and Castle (1927) accomplished the difficult measurement of the gas exchange of blood from a cat's muscle isolated except for circulatory connection. The average R. Q. was 0.71, compared with an average of 0.80 for the expired air of the same animal. They concluded that muscle could oxidize other substances than carbohydrate and was not different from the entire animal in this respect.

In some recent experiments Richardson, Shorr and Loebel (1930) report the successful maintenance of excised mammalian muscle with a rate of O_2 consumption apparently normal and no appearance of degeneration. The R. Q., as determined in an apparatus of Barcroft type, was usually well below one and in the range expected for the animal as a whole.

There was a final rise in R. Q. in eviscerated animals kept till they became

moribund which suggested that *in extremis*, utilization of carbohydrate was favoured. The conspicuous condition of the moribund preparation is the low O_2 tension of its blood, and the low O_2 tension might be incompatible with fat oxidation and therefore favourable to the predominant use of carbohydrate. Study of the acid-base situation *in extremis* does not show the reliability of the quotient, for the O_2 consumption has already fallen so low that very small changes in CO_2 reserves have a great influence on the R. Q. The hypoglycemia which develops after evisceration is against the chances for a facultative diversion of oxidation in terminal asphyxia from partly fat to exclusively carbohydrate. Within an hour after evisceration the blood sugar was usually down to 60 mgm. per cent, as shown in table 1, and kept slowly declining for several hours

TABLE 2

Respiratory quotient of eviscerated, decapitated cats

DATE	TIME	CO_2	O_2	R.Q.	R.Q. BEFORE EVISCERATION	MUSCLE CO_2 CHANGE	R.Q. CORRECTED FOR MUSCLE
	minutes	*cc.*	*cc.*			*per cent*	
November 7	124	658	854	0.77	0.72		
November 26	78	481	610	0.79	0.82		
November 28	113	1,323	1,520	0.87	0.74		
February 19	188	1,243	1,620	0.77	0.77	0	
February 24	184	1,473	2,070	0.71	0.63	0	
February 26	119	962	1,150	0.84	0.65	0	
March 3	147	952	1,212	0.78	0.60	0	
March 5	150	845	984	0.86	0.77	+3	0.91
March 10	270	1,584	1,916	0.83		+3	0.85
March 12	205	1,452	1,771	0.82		0	

while the true R. Q. of metabolism did not change. The blood sugar level was lowest when the rise in R. Q. appeared.

During and immediately after evisceration there was usually a marked hyperglycemia. The immediate period of evisceration usually had a high R. Q., but it was likewise associated with depletion of CO_2 reserves. Consequently we have no fair criterion as to whether an excess of circulating blood sugar in the evisceration period favours increased utilization of carbohydrate. The next series of experiments was designed to test the effect of distinct hyperglycemia on the gas exchange.

The R. Q. during administration of extra glucose. To test the effect of sugar administration on the R. Q., glucose was infused continuously into the saphenous veins of decapitated, eviscerated cats, in amounts increasing in successive experiments. As control for the validity of the R. Q. measured, arterial blood and muscle CO_2 contents were determined as before.

One experiment is summarized graphically in figure 4. As the blood sugar content rises the R. Q. also rises, but on account of the progressive decrease in O_2 consumption, the average R. Q. for 222 minutes after evisceration is only 0.82. The acid-base equilibrium was controlled by examination of arterial blood alone, which steadily lost CO_2. But as it lost in total CO_2, its capacity diminished correspondingly, so that the CO_2 tension remained about constant, implying stability of the CO_2 reserves.

Another experiment is described in the protocol of table 3. The average R. Q. is only 0.82 during 198 minutes of hyperglycemia after evisceration, showing its most extreme rise to 0.93 only when the blood sugar fell below

TABLE 3

Eviscerated cat with sugar. February 17, 1930

4 grams glucose injected per saphenous vein

	TIME	VENTILATION RATE	O_2	O_2	CO_2	R.Q.	ARTERIAL BLOOD				SUGAR	MUSCLE CO_2
							CO_2	O_2	Combining power in 5.5 per cent CO_2 in O_2			
									CO_2	O_2		
	minutes	*cc. per minute*	*cc.*	*cc. per minute*	*cc.*		*cc. per 100 cc.*	*cc. per 100 cc.*	*cc. per 100 cc.*	*cc. per 100 cc.*	*mg. per 100 cc.*	*cc. per 100 grams*
I. Decapitated	43	560										
II	35	700	856	24.5	646	0.75	42.2				200	
III	39		890	22.8	685	0.77	42.5	11.9	44.0	14.0	185	23.4 24.8
IV Post evisceration	42		407	9.70	429	1.05	35.5	12.6			192	
V	50	400	570	11.4	455	0.80	38.5	14.3			272	
VI	60		524	8.74	426	0.81	38.6	13.0			170	
VII	50		404	8.08	321	0.80						
VIII	38		241	6.34	223	0.93	36.8	12.1	36.8	13.4	077	30.0 31.4

normal. The failure of any appreciable rise in R. Q. is not the result of under-ventilation, for the O_2 consumption remained normal, the arterial blood was well oxygenated, and its CO_2 tension did not change appreciably. Nevertheless the muscles gained CO_2, and the amount of CO_2 retained in the muscles is $0.06 \times 1200 = 72$ cc. When added to the CO_2 expired during the 198 minutes after the evisceration period, the R. Q. becomes 0.86.

Five experiments are summarized in table 4, showing an average R. Q. of 0.84, considerably greater than the usual R. Q. of decapitated cats. In these experiments the blood sugar was maintained at from 200 to 500 mgm. per cent. Although the apparent R. Q. did not rise to the level of exclusive carbohydrate metabolism, several changes showed that the extra

sugar exerted a pronounced influence on metabolism. In the general condition of the animals there was a decided improvement in blood pressure. There is no definite improvement in O_2 consumption shown, the average being about the same as for eviscerated cats without sugar. The CO_2 content and tension of arterial blood were not so much depleted. Lastly, the muscle CO_2 content in two cats increased. These changes are not attributable to under-ventilation, as judged by the sustained O_2 consumption and O_2 saturation of arterial blood and absence of asphyxial signs. The inference is that, while administration of great excess of glucose does not significantly divert the course of metabolism toward exclusive use of carbohydrate, it nevertheless notably diminishes the liability of over-ventilation. Glucose administration either reduces the tendency toward acidosis or increases the CO_2 capacity of the tissues. This effect appears to be more significant than the increased oxidation of carbohydrate.

TABLE 4

Eviscerated cats with sugar

DATE	TIME	O_2	CO_2	R.Q.	MUSCLE CO_2 CHANGE	CO_2 LOST OR GAINED	R.Q. CORRECTED	GLUCOSE INJECTED
	minutes	*cc.*	*cc.*		*per cent*	*cc.*		*grams*
November 21	226	2,145	1,827	0.85				0.92
December 31	163	1,566	1,446	0.92				2.8
January 16	222	1,206	986	0.82				3.6
February 17	198	1,739	1,425	0.82	+6	72	0.86	4
March 17	192	1,195	1,023	0.81	+3.3	52	0.86	8
Average				0.84				

Burn and Dale (1924) comment on the disappearance of more sugar than could be oxidized, even assuming an R. Q. of one. In our last experiment (March 17), the 1200 cc. of O_2 consumed would be only equivalent to the oxidation of 1.5 gram of carbohydrate, leaving 6.5 grams of the infused carbohydrate to be accounted for. The weight was 2500 grams after evisceration and decapitation, and the final blood sugar content 545 mgm. per 100 grams. We cannot estimate the distribution of fluid in which sugar is similarly dissolved, but to account for the remainder in simple solution in the body fluids at equal concentration with the blood would require an allowance of 1200 cc., half of the body weight, without allowing for the presence of any original sugar. It is evident that much of the sugar aside from that oxidized must have passed into the muscle.

Burn and Dale (1924) proposed that O_2 consumption by the eviscerated preparation was a matter regulated by the blood sugar content. Bornstein (1929) reported a rise in R. Q. of eviscerated dogs after glucose administra-

tion and concluded that while muscle is not obligated to the exclusive use of carbohydrate, it would turn to it when abundantly present. Markowitz (1928) found that surgically eviscerated dogs used some sugar, as judged by R. Q.s between 0.85 and 0.90, but did not observe that extra injected sugar raised the R. Q.

Our experiments show that there is a slight increase in the proportion of sugar oxidized during marked hyperglycemia. The extra use of sugar is accompanied by the disappearance of a much larger amount than could have been oxidized, and an increase in the CO_2 content of the tissues.

In order to follow the effects of the utilization of sugar further, we next supplied eviscerated cats with insulin and sugar in massive doses. If insulin facilitates the utilization of carbohydrate by muscle, the muscles should show in more pronounced fashion the gain in CO_2 content and reduction of the susceptibility toward over-ventilation which characterized the preparations receiving glucose alone.

The R. Q. during administration of insulin and glucose. The next experiments were designed to show the R. Q. of eviscerated cats receiving insulin and sugar. Markowitz, Mann and Bollman (1929) have remarked on the massive doses of insulin and glucose necessary to induce glycogen deposition in dogs without liver or pancreas. In order to accentuate the effect we have used solutions of 20 per cent glucose containing about 80 units of insulin in 60 cc., infused through the saphenous vein at about 12 cc. per hour. Soon after the infusion started the condition of the preparation improved. The blood pressure and reflexes became better, and the rate of O_2 consumption was often greater in the hour after the start of insulin and glucose. During the first period after evisceration and before insulin and glucose infusion, blood and muscle samples were removed and the cats were subsequently maintained for a further three hours or more with periodic removal of blood and muscle.

The results of a typical experiment are shown in figure 5. The observed R. Q. was 0.81, and the diminishing CO_2 content of arterial blood was approximately balanced by the decline in CO_2 capacity, so that the alveolar CO_2 tension only fell slightly. The muscles, however, gained on the average 5.7 volumes per cent of CO_2. Taking muscle as 50 per cent of the body weight before evisceration, the amount of CO_2 retained in muscle is $0.057 \times 1350 = 77$ cc. Adding that to the CO_2 expired raises the R. Q. to 0.89, quite a significant change. The change in muscle CO_2 content is not the result of dehydration, for the proportion of muscle dry substance did not change.

In table 5 are summarized the results of eleven experiments. The average R. Q. with insulin and sugar is 0.88, and when corrected for the amount of CO_2 retained in the muscles the average R. Q. of eight experiments is 0.94.

The corrections for muscle CO_2 retention are approximations based on the average gain in from three to five muscle pairs. In only one case is the corrected R. Q. greater than one, and in that only 1.02. The corrected R. Q. indicates that carbohydrate is the principal substance oxidized under the influence of large doses of insulin and sugar.

It is also important to determine whether CO_2 retention is peculiar to the muscles alone or whether it is only representative of CO_2 retention by the other tissues present, which still comprise some 40 per cent of the eviscerated organism. In studying the sources of the CO_2 blown off from eviscerated cats by over-ventilation, Ferguson, Irving and Plewes (1929) found that only some 20 per cent of the total amount lost came from

TABLE 5

R.Q. of eviscerated cats receiving massive injections of insulin and sugar

DATE	TIME	R.Q.		MUSCLE CHANGE	R.Q. CORRECTED
		Before eviscera-tion	After eviscera-tion		
	min.			*vol. per cent CO_2*	
January 20	185	0.71	0.90		
January 27	160	0.64	0.90		
January 29	216	0.79	0.83		
February 10	155	0.74	0.80	+9	0.92
March 18	213		0.78	+10	0.89
March 23	258		0.92	+8.5	1.00
April 1	272		0.86	+4.2	0.89
April 7	183		0.81	+6.0	0.89
April 8	186		0.94	+5.7	1.02
April 28	205		0.91	+2.5	0.95
May 19	170		0.91	+4.3	0.95
Average			0.88	6.9	0.94

muscles. Likewise when CO_2 was retained by intact cats breathing CO_2 rich mixtures, only about 20 to 30 per cent of the whole amount retained could be located in the muscles (Irving, Ferguson, and Plewes, 1930). If the CO_2 retention of muscles represents only one fifth of the total amount retained under the influence of insulin and sugar, the correction of CO_2 added to the R. Q. should be about four times as great as the amount of CO_2 retained by muscle alone. The corrected R. Q.s would then be considerably greater than one.

In order to determine whether CO_2 retention is peculiar to muscle, we next increased the rate of ventilation after the start of insulin and sugar administration. It was found that considerable over-ventilation was necessary to prevent CO_2 retention by the muscles. In the first experi-

ment listed in table 6 the stroke volume of the pump was increased about 30 per cent, and in the second about 50 per cent. The venous blood CO_2 tension decreased very little, but the muscles showed the spontaneous twitching and exaggerated excitability characteristic of over-ventilation. Nevertheless the muscles still gained some CO_2. In the last three experiments the ventilation per stroke was doubled, and the muscles lost CO_2 slightly. In correcting these R. Q.s it is necessary to allow for CO_2 withdrawn from the venous blood. Under ordinary ventilation the blood CO_2 change was not significant toward the R. Q., but during over-ventilation the blood CO_2 loss may be one-third of the muscle loss. Making appropriate correc-

TABLE 6

Effect of overventilation on muscle CO_2 content and consequent correction of the R.Q.

DATE	TIME	R.Q. OBSERVED	MUSCLE CHANGE	R.Q. CORRECTED
	minutes		*vol. per cent CO_2*	
April 28	205	0.91	+2.5	0.95
April 30	272	0.90	+2.6	0.91
May 14	219	0.86	−3.2	0.82
June 17	144	1.02	−3.5	0.92
June 19	130	1.19	−4.0	1.03
Average				0.93

TABLE 7

CO_2 retention by the muscles of decapitated cats and its influence on the R.Q.

DATE	TIME	R.Q. OBSERVED	MUSCLE GAIN	R.Q. CORRECTED
	minutes		*vol. per cent CO_2*	
April 14	192	0.71	+8	0.79
April 15	190	0.75	+4	0.76

tions for CO_2 lost or gained brings the corrected R. Q.s within the range of the corrected R. Q.s at ordinary rates of ventilation. The average R. Q. corrected for over-ventilation is 0.93, compared with 0.94 when corrected for muscle CO_2 retention. If extra CO_2 had been withdrawn from or retained by other tissues, the correction during muscle CO_2 loss or retention should have been about four times greater and the corrected R. Q.s determined by the two methods would have been entirely discordant. As it is, their close coincidence is good evidence that the CO_2 changes are peculiar to the muscles and that the method of allowance for changes in muscle CO_2 is correct.

The tendency of muscle to retain CO_2 under the influence of insulin and sugar is not restricted to eviscerated animals. It still occurs to an extent sufficient to modify the R. Q. of ordinary decapitated cats, as is shown in table 7.

That the insulin and sugar were causing the characteristic deposition of glycogen observed by Best, Hoet and Marks (1926) was shown by increased glycogen content of the muscles in four experiments. The general improvement of the animals has already been described as a characteristic effect.

The increased CO_2 content of muscles during the active metabolism of carbohydrate seems to represent increased CO_2 capacity rather than the effect of increased CO_2 tension. If we consider that the CO_2 tension of muscle is not far from the tension in venous blood (Krogh, 1922), there is no evidence in the venous blood examined for a tension increase in the muscle greater than 20 mm. Lacking a complete dissociation curve for mammalian muscle, we can only refer to the figures of Fenn (1928) for frog muscle. The normal CO_2 content at 20 mm. tension was 22 cc. per 100 grams. In order to increase the content to 27 cc., tension must increase to 80 mm. Now the average gain in muscle CO_2 content shown in table 5 is 6.9 cc., so that if there is a resemblance between the buffering capacity of frog and mammalian muscle, the CO_2 tension would need to be quadrupled to allow it to account for the gain in CO_2 content which occurs during carbohydrate utilization. It is hardly possible that the buffering capacity of mammalian muscle is sufficient to allow the observed increase in CO_2 content to be attributed to change in CO_2 tension.

Under the influence of insulin and sugar the R. Q. of the eviscerated cat shows considerable diversion of metabolism toward the oxidation of carbohydrate. When corrected for the amount of CO_2 retained in muscle, the R. Q. approaches but never exceeds one. The change in CO_2 content is peculiar to muscle, as was shown by the agreement between corrected R. Q.s during muscle CO_2 retention and loss from over-ventilation. The usual characteristic effects of insulin and sugar, i.e., improved circulation and general condition and glycogen storage, demonstrate the activity of insulin in the preparation. The gain in muscle CO_2 is a further characteristic of the action of insulin and sugar, which appears to rest on increased CO_2 capacity of the muscles themselves. The problem of the cause of the increased CO_2 content will be discussed with its appropriate evidence in another communication.

SUMMARY

After evisceration of a decapitated cat there is a tendency toward over-ventilation. When the ventilation is reduced, the preparation may be kept in a state of balanced CO_2 equilibrium with R. Q. near 0.80. The

balance of the CO_2 equilibrium can only be controlled by establishing the fact that the CO_2 content of muscles does not change. Arterial and venous blood are not reliable indicators of CO_2 balance in the eviscerated preparation. The R. Q. is not much greater than that of the entire animal, and shows that resting muscle oxidizes about the same substances.

Intravenous administration of glucose causes a slight elevation of R. Q. to an average level about 0.85. The tendency toward over-ventilation is relieved, and the muscles even gain CO_2.

With massive doses of insulin and glucose the tendency toward CO_2 retention in the muscles is more marked. The average observed R. Q. was 0.88, and the corection for CO_2 retained in the muscles raised the R. Q. to 0.94, indicating diversion of oxidation to the principal use of carbohydrate. The correction of the R. Q. for the amount of CO_2 lost from muscle during slight over-ventilation indicates that only muscle is appreciably altered in CO_2 content. The action of insulin and glucose, in addition to increasing carbohydrate oxidation and glycogen storage affects the CO_2 equilibrium of muscle, probably by increasing its capacity.

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SOME OBSERVATIONS UPON CHANGES IN VOLUME FLOW OF BLOOD ACCOMPANYING CHANGING RESPIRATORY CONDITIONS

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The observations upon the volume flow of blood which are reported in this paper are a continuation of experiments which have been described in earlier papers (Bronk and Gesell, 1927; Bernthal, Bronk, Cordero and Gesell, 1928). The purpose of these studies, as stated in the preceding reports, is an attempt at a closer correlation of and determination of the mechanisms of circulatory and respiratory control. The functional activities of circulatory and respiratory systems being as closely interlocked as they are, it follows as an obvious fact that any conception of the controlling factors of one, under a given set of conditions, is incomplete unless there is taken into consideration the changes which have simultaneously occurred in the other under the same conditions.

From the standpoint of the direct influence of circulation upon respiratory control, it is most essential to know what the changes in blood flow are. However, it is also desirable to understand the mechanisms whereby these changes are brought about, because in two systems having a common function there may very well be similarities in the factors which control them, and information concerning one may throw light upon the mechanisms of the other.

It has been the aim of these experiments, then, to formulate a composite picture of blood volume flow changes occurring over the body as a whole under conditions which modify respiratory response, and, wherever possible, to determine the mechanisms responsible for the changes. To do this, the volume flow in several different arteries has been recorded. For the purposes of discussion, these arteries may arbitrarily be considered as supplying three general regions of the body which are fundamentally different from the standpoint of general function and from the standpoint of circulatory adjustments. These regions are the head, in which the blood flow changes have been shown (Bernthal, Bronk, Cordero and Gesell, 1928) to be dominated by brain flow, the abdominal visceral region, and

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what may be referred to as "general peripheral regions" including neither brain nor viscera but rather muscle, nerve, bone, connective tissue and skin and represented in these experiments by the hind or the fore limb. Results of measurements of common and internal carotids, vertebral and femoral arteries have already been reported. This paper gives further data upon femoral and brachial arterial flow, upon superior mesenteric arterial flow, which it was hoped would reflect general splanchnic flow, and a few determinations of total volume flow as measured at the arch of the aorta.

METHODS AND PROCEDURE. Volume flow measurements were made by the thermo-electric method of Gesell and Bronk (1926), which provides a continuous graphic record of the changes. Quantitative determinations of flow were not made, indications of directional changes with a rough idea of their extent adequately serving the purpose of the experiments.

The experimental animals were dogs, anesthetized with morphine and urethane and heparinized to prevent the clotting of blood. The experimental conditions used were confined to those which are known to modify respiration. These conditions were:

1. Decreased alveolar oxygen pressure.
2. Impairment of the oxidative mechanism of tissue cells by intravenous sodium cyanide injection.
3. Increased alveolar oxygen pressure.
4. Increased alveolar carbon dioxide pressure.

Gaseous mixtures were administered with rebreathing tanks, using normal ventilation with the chest intact or constant artificial ventilation with pneumothorax. Sodium cyanide was given by intravenous injection. Blood pressure was allowed to fluctuate spontaneously or was artifically kept at a constant mean level by attaching a reservoir filled with blood or glucose-saline solution to the lower end of the abdominal aorta.

RESULTS. *A. Femoral and brachial arterial flow.* Typical responses of femoral arterial flow to the experimental conditions outlined have already been reported, but no attempt at distinguishing between central nervous and local effects was made. In the present series of experiments we have attempted to evaluate these changes more carefully, distinguishing between changes dependent upon purely local factors in the tissues whose flow was being measured, those involving central nervous vasomotor influences and those which were merely the result of changes in driving head of arterial blood pressure. To do this, the flow of blood in each of a pair of arteries was simultaneously recorded, the nerve supply of the region supplied by one artery of a pair being intact and that of the other artery having been destroyed. The simultaneously recorded changes were then compared, using the flow in the innervated tissue as control. The femoral arteries were used in the earlier experiments. In later

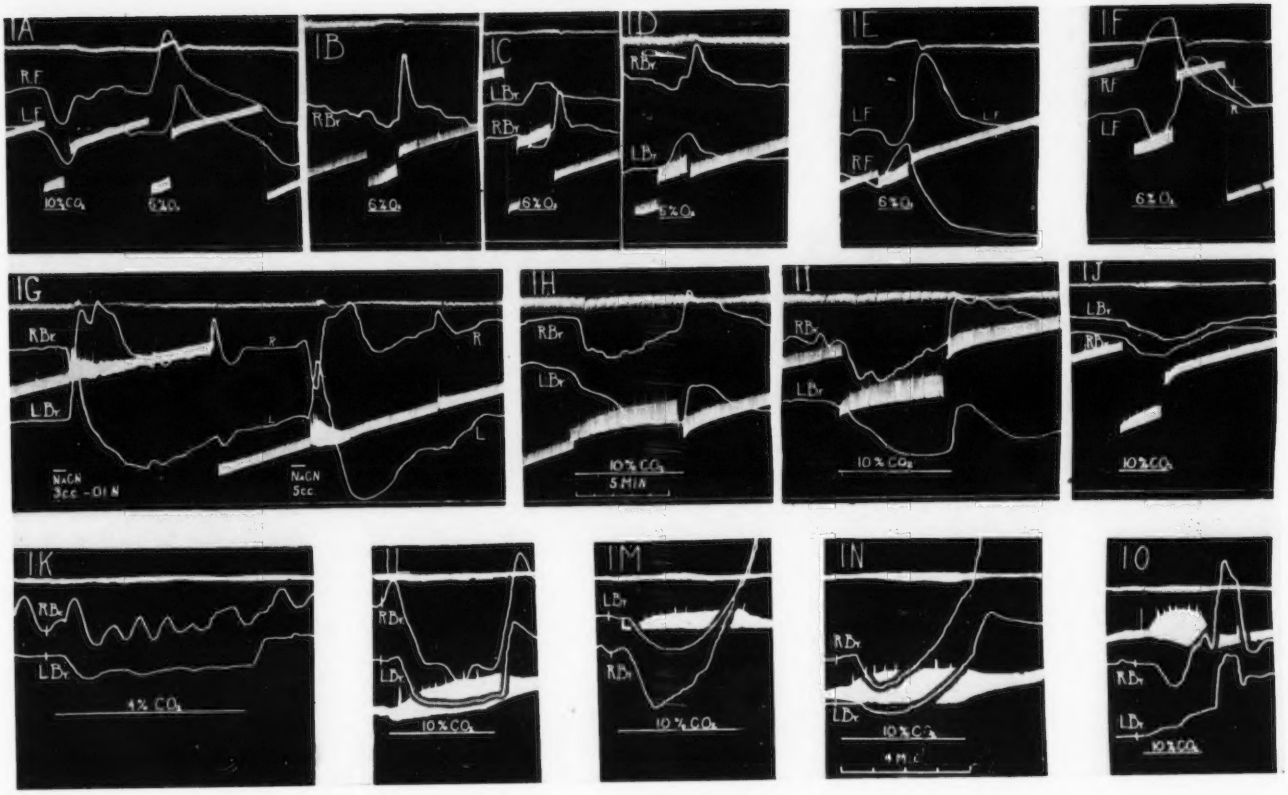
1A
RF
LF
10%CO2
6%O2
1B
RBr
6%O2
1C
LBr
RBr
6%O2
1D
RBr
LBr
6%O2
1E
LF
RF
6%O2
1F
RF
LF
6%O2
1G
RBr
LBr
NaCN
3cc - 01 N
NaCN
5cc
1H
RBr
LBr
10% CO2
5 MIN
1I
RBr
LBr
10% CO2
1J
LBr
RBr
10%CO2
1K
RBr
LBr
4% CO2
1L
RBr
LBr
10%CO2
1M
LBr
RBr
10% CO2
1N
RBr
LBr
10%CO2
1O
RBr
LBr
10%CO2

Fig. 1

experiments the brachial arteries were used because of the greater ease in securing complete denervation, it having been previously demonstrated that the responses in the brachial and femoral arteries are identical in their nature.

Before section of the nerves in each experiment a series of records was made with the nerve supply of each leg intact in order to be sure that the responses in each of the arteries were really comparable under identical conditions. Figure 1A illustrates such a record and demonstrates how accurately the flows correspond.

1. *The effects of low alveolar oxygen pressure and of sodium cyanide injection.* The most common response to low alveolar oxygen may be seen in figure 1B representing flow in the right brachial artery before nerve section. Here there is an initial decreased flow almost immediately upon administration of low oxygen. This is followed by an increasing flow throughout the remainder of the administration. Almost immediately upon readministration of room air there is a further very sharp and often very large increase in volume flow, which is followed by a decrease which is quite as rapid for a time and then slowly gives way to complete recovery. The sharp rise upon readministration of room air we have called the "post-administrational increase" in volume flow. During the taking of the record represented by figure 1B the mean blood pressure was artifically maintained at virtually a constant level, indicating that the changes described occurred independently of changes in driving head of pressure.

The results of removing the influence of the central nervous system are shown in figures 1C, 1D, 1E, and 1F taken from several different experiments in which either the right brachial plexus or the left femoral, sciatic and obturator nerves were severed. Using figure 1C or 1D as an example, the differences between flow to the innervated limb (curve *R Br.*) and the flow in the denervated limb (curve *L Br.*) upon the administration of 6 per cent oxygen are at once apparent. Whereas the response in the right leg (*R Br.*) is the usual one with initial decreased and post-administrational increased flow, the response in the left limb has been strikingly modified. There is no initial decrease in flow. The first effect of the low alveolar oxygen is an increasing volume flow which persists throughout the period of administration. Upon readministration of room air this increased flow is not followed by a further post-administrational increase but gives way immediately to a *decreasing* flow until the preadministration level has been reached. The same results can be seen in figures 1E and 1F, and they occur regardless of whether or not the mean blood pressure is allowed to fluctuate.

These experiments seem to indicate, then, that of all of the various changes in "general peripheral flow" noted upon the administration of low

alveolar oxygen pressures, only the late increase noted during administration is due to the local effects of deficient oxygen supply. The initial decrease in flow and the post-administrational increased flow are apparently vasomotor effects which are dependent upon the integrity of the nerve supply to the tissue and in all probability have their origin in the central nervous system.

There are, then, apparently two effects constantly at work in the control of volume flow in this region, one central nervous and the other peripheral. In the case of low oxygen administration at least, these effects seem to be opposite in their results, and of differing sensitiveness, the central effect always appearing first. Thus, during administration of low oxygen we see the central vaso-constricting effect appearing first and gradually being replaced by the peripheral dilating effects of low oxygen. Upon readministration of room air we see first vaso-dilatation of central origin which gives way to the local constricting effects of recovery from low oxygen.

These experiments do not, of course, indicate upon what effect of low oxygen these reactions are dependent. They are not contrary, however, to a possibility tentatively suggested in an earlier paper (Bernthal, Bronk, Cordero and Gesell, 1928) in which it was proposed that they might be due to the acid effect of deficient oxidation, the administrational decrease in flow being due to central acid stimulation of vasomotor centers, the administrational increase to the local relaxing effects of acid upon the contractile elements of the vessel walls and perhaps also to a direct effect of lack of oxygen, the post-administrational increase to increasing alkalinity of vasomotor centers coincident with readministration of room air, and the post-administrational decrease to the local effects of increasing alkalinity of the peripheral tissues as well as recovery from the direct effects of lack of oxygen.

In interpreting the peripheral chemical mechanism for volume flow control there is also the possibility (Gesell, 1929) that the functional activity of the contractile elements is modified by accumulation of their own metabolic products as well as by chemical agencies transmitted to them from the surrounding tissues or carried to them from other tissues by the blood. Thus, during the administration of low oxygen, the deficient oxidations within the cells of the contractile elements may lead to accumulation of endogenous acid, the reaction which results augmenting and perhaps preceding the reaction due to acid arising from sources outside the cells.

The effects of sodium cyanide injection, as seen in a limited number of experiments, may also be in harmony with the existence of a central and peripheral acid mechanism of volume flow control. Upon injection of cyanide the innervated leg (*R Br.* in fig. 1G) exhibits the usual reaction, namely, an initial decreased flow followed rapidly by an increase far above

preadministration levels. The denervated leg (*L Br.*), on the other hand, shows an immediate increase in flow which gives way to recovery and often to a decreased flow. Here again the central constricting effect might be interpreted as acid stimulation and the peripheral relaxing effect to the local action of acid accumulation. (Whether the late decrease in flow in the denervated limb is dependent alone upon blood pressure changes or can be attributed to other causes remains to be seen.)

One more observation may be pertinent at this point in view of the differences observed in earlier reports between brain flow and flow to the limb upon administration of low oxygen mixtures. It will be recalled that the flow to the brain, in contrast to the complex changes in femoral flow, responded to low oxygen by a simple smooth rise. A comparison of the response of denervated tissue with that of the brain flow will reveal a striking similarity. In other words, the response of the brain flow to administration of low oxygen is apparently more like that of denervated tissue than it is like that of tissue whose vasomotor nerve supply is intact.

2. *The effects of high alveolar carbon dioxide.* The vaso-constricting effect of carbon dioxide when administered to animals with nerves intact is well known, and its effect in decreasing femoral blood volume flow has been demonstrated by Bronk and Gesell (1927). The mechanism controlling this vaso-constriction is generally conceded to be the acid effect of carbon dioxide causing stimulation of the vaso-constrictor centers (Mathison, 1911). The peripheral vasomotor action of carbon dioxide, on the other hand, has been shown by Bayliss (1901), Hooker (1912) and others to be one of vaso-dilatation and this has been interpreted as a local effect of increased hydrogen concentration. Upon the basis of these conceptions, it was expected that the flow to the denervated limb in our experiments would be increased upon the administration of high alveolar carbon dioxide.

Typical effects of the administration of 10 per cent carbon dioxide mixtures are shown in figures 1H, 1I and 1J where it will be seen that there is a decreased flow in the denervated leg (curve *L. Br.*) as well as in the innervated leg (curve *R Br.*). The only differences to be noted are a more gradual change in the denervated leg in some of the experiments and sometimes a smaller extent of change. Apparently there is some factor at work here other than the local dilating effect of increased hydrogen ion concentration. The possibility that denervation was incomplete was obviated by completely amputating the limb so that its only connection with the trunk was the fluid one provided by the blood stream passing through connecting cannulae. The results were unaltered by this procedure.

These experiments do not provide an explanation of the response of the volume flow to high carbon dioxide in the denervated limb. There are several possibilities which may be considered, however. It was

thought that the decreased flow might be the result of increased viscosity of blood, for administration of carbon dioxide leads to passage of water from plasma into corpuscles and into tissue spaces. To test this possibility, the corpuscular volume was followed by hematocrit determinations through several experiments. Changes which are representative of all of the experiments are as follows:

Corpuscular volume

Pre-administration per cent	During (10% CO_2) per cent	Post-administration per cent
53.0	54.5	52.5
43.0	45.0	
42.0	43.0	

While the changes in corpuscular volume are definite, they hardly seem large enough to explain the decreased volume flow on the grounds of increased blood viscosity alone. Another objection to increased blood viscosity as an explanation is that it might be expected to affect the flow in other parts of the body in the same way. Yet brain flow is increased upon administration of high carbon dioxide as is also the flow in the superior mesenteric artery, regardless of whether or not the nerve supply is intact.

Another possible explanation is stimulation of the suprarenal glands by carbon dioxide (Anrep, 1913). Itami (1913) observed a decrease in the volume (plethysmograph) of the denervated forelimb upon the administration of 10 to 12 per cent carbon dioxide, and accounted for his results upon the basis of increased liberation of epinephrine into the blood stream. This explanation seemed to apply equally well to the results upon volume flow of blood until measurements were subsequently made of the volume flow in the superior mesenteric artery. Here the effects of carbon dioxide administration are an *increase* in volume flow which occurs independently of blood pressure changes. If sufficient epinephrine is liberated into the blood by carbon dioxide administration to cause vasoconstriction in the fore limb, the same effect upon the region supplied by the superior mesenteric artery might be expected.

The apparent lack of harmony between these results and those of Bayliss and of Hooker becomes less serious upon further analysis of the respective data. It will be recalled that Hooker's and Bayliss' data are taken from experiments in which vessels were perfused with Ringer's solution or in which vascular rings were suspended in Ringer's solution. These are obviously experimental conditions quite widely different from those of the volume flow experiments in which the limb was circulated with whole blood which had passed through all of the normal channels of the body. That the reaction of the vessels to carbon dioxide can be altered by differences in experimental conditions is evidenced by figures

1K to 10 which represent carbon dioxide records taken at various stages of a prolonged experiment. The first records, 1K and 1L, taken within two hours after nerve section show the usual marked decrease in flow in each limb, and this is sustained throughout the administration in each case. As the experiment progresses, the initial decreased flow gives way eventually to an increasing flow during administration of carbon dioxide (fig. 1M). As the experiment is continued still further, the period of decreased flow during administration of carbon dioxide becomes progressively shorter and the period of increased flow relatively longer (fig. 1N) until in figure 10, six hours after section of the nerves, the denervated leg finally responds to the carbon dioxide by a simple rise in flow, which would be the expected result if the vessels were reacting in accordance with the conclusions of Hooker. Apparently, then, the change of experimental conditions coincident with the progressive deterioration of the animal during a prolonged experiment is sufficient to bring about a diametrically opposite local vasomotor reaction to carbon dioxide administration. It is hardly to be expected, therefore, that the results in these experiments should necessarily coincide with the results of perfusion experiments in which experimental conditions may be widely different.

One more purely hypothetical possibility in the explanation of the decreased flow in the denervated leg upon administration of high carbon dioxide suggests itself at this point. Obviously, there are two potential effects of carbon dioxide upon peripheral vasomotor reactions,—one, the more familiar relaxing effect upon vascular contractile elements, the other a constricting effect the mechanism of which is less well defined, but which seems to dominate when the environment of the tissues is more nearly normal. It has been shown that one of these effects can merge into the other. Can it be that carbon dioxide is acting in some other way than by increasing the hydrogen ion concentration or that it is acting upon some structures other than the vascular contractile elements (such as specialized nerve endings or ganglion cells) and that as the tissues deteriorate during an experiment these structures gradually lose their irritability until finally their action is overcome by the direct relaxing effect of carbon dioxide upon the contractile elements of the vessels themselves? This is admittedly hypothesis only, and it will be alluded to again later in the discussion.

B. *Superior mesenteric arterial flow.* Volume flow measurements in the superior mesenteric artery were undertaken with the object of gaining some idea of the circulatory reactions of the abdominal viscera to the experimental conditions outlined. There is, however, considerable doubt, as will be shown, as to whether the reactions of the abdominal viscera in general are accurately reflected by those in the distribution of the superior mesenteric artery. Dissection of the artery entailed some degree of

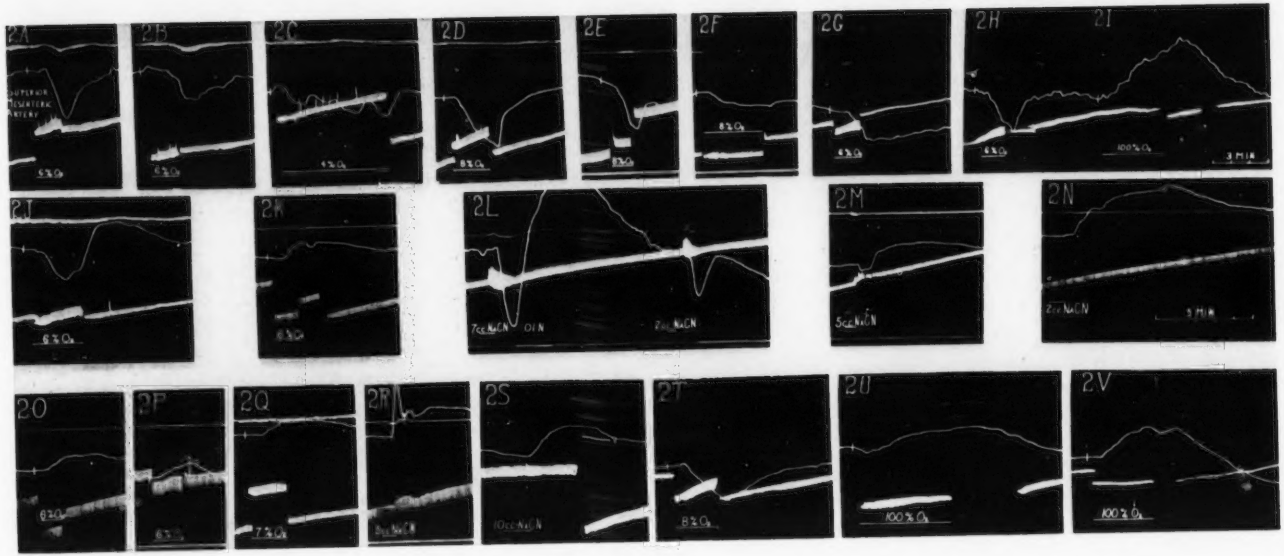

Fig. 2

destruction of the superior mesenteric nerve plexus. However, the functional integrity of the plexus could be verified by observing the effect upon the volume flow of electrical stimulation of the splanchnic nerves.

In general, the results obtained in measurements of superior mesenteric arterial flow were less clear cut than those in other regions of the body, there being in many cases spontaneous rhythmic changes in flow which complicated and sometimes masked the results of the experimental procedures. In addition, the changes in flow were extremely sensitive to small changes in mean blood pressure, a single example of which may be seen in figure 3A in which every slight wave in blood pressure has its corresponding wave in blood flow. In the experiments in which denervation was performed, accurate interpretation of the results was made more difficult by the lack of a comparable region whose nerve supply could be left intact for the taking of simultaneous control reactions in innervated tissue.

1. *The effects of low alveolar oxygen pressures and of injection of sodium cyanide.* Typical effects of low alveolar oxygen pressure upon flow in the superior mesenteric artery are seen in figures 2A, 2B and 2C in which the administration of room air is interrupted by 6 and 14 per cent oxygen mixtures. After a delay of a minute or more during which it rises slightly, the flow decreases rather sharply, remains so during the administration and returns to preadministration levels upon readministration of room air. In each of the experiments from which these records are taken, the nerve supply to the abdominal organs was intact, the chest was intact so that ventilation was under the control of the animal and blood pressure was allowed to vary spontaneously.

That the changes are not dependent upon blood pressure variations is shown by figure 2D in which the mean blood pressure is artifically kept at a constant level during the administration of 8 per cent oxygen. The only differences to be seen as a result of a constantly maintained blood pressure are an absence of the slight initial increase in flow and a more prompt onset of the decreased flow, indicating that in figures 2A and 2B the initial increase in flow and the delay in the onset of the decreased flow are effects of the temporary rise in blood pressure which usually occurs at the beginning of a low oxygen administration.

The same directional changes are noted when low oxygen is administered during artificial ventilation. The significant difference between administration during artificial and normal ventilation is to be seen, however, in the *extent* of the response, the decrease being much sharper and more pronounced during constant artificial ventilation. This is well illustrated by figures 2E and 2F, taken from the same experiment, 8 per cent oxygen having been administered in each case, the first with constant artificial ventilation and the second with normally increasing ventilation. The

difference may lie in both the greater impairment of oxidations and the less complete washing out of carbon dioxide in the vaso-constrictor centers in the absence of compensatory hyperpnea during the artificial ventilation.

As was the case in the flow in the femoral artery (Bernthal, Bronk, Cordero and Gesell, 1928), there is a great variability in the response to low oxygen. In many instances, as exemplified by figure 2G, there is an incomplete or a very slow recovery from the decreased flow. This type of response is seen most frequently in experiments in which the blood pressure is allowed to change spontaneously. Its dependence upon blood pressure changes is indicated by the response seen in the administration immediately following (2H) in the same experiment in which a constant mean blood pressure was maintained and a complete and prompt recovery is seen to have taken place.

Another interesting variation in response is seen in figure 2J in which the initial decreased flow is seen to give way during administration of low oxygen to an increasing flow which is followed by a post-administrational increase far above pre-administration levels, much the same as is seen in the femoral and brachial flow.

In one experiment (fig. 2K) there was a rise in flow occurring independently of blood pressure changes during the administration of 6 per cent oxygen. The explanation of this exception to the usual effect of low oxygen administration is not apparent.

The effects of sodium cyanide injection are shown in animals whose nerve supply is intact in figures 2L, 2M and 2N. In 2L the blood pressure is allowed to change and in 2M and 2N it is maintained at a constant level. The more typical response is seen in figures 2L and 2M,—an initial decreased flow followed by a rather prolonged increased flow with eventual recovery. In figure 2N the initial decreased flow is absent. Curiously enough, this record is taken from the same experiment as that in which the exceptional response (increased flow) to low oxygen was noted. (There were, however, instances in a few other experiments in addition to this one in which there was absence of the initial decreased flow.)

Without discussing all of the possible explanations of the mechanism for the volume flow responses of the superior mesenteric artery to low oxygen and sodium cyanide injection, it is pertinent, in view of the earlier discussion, to observe that they are capable of the same tentative interpretation as were the responses of femoral and brachial artery. The fact that increased flow during administration of low oxygen is not a common occurrence in the superior mesenteric artery does not necessarily mark the response as being essentially different from that of the femoral, for differences in richness of the vascular bed or differences in local metabolic rate may lead to lessened impairment of oxidations and lessened rate of

peripheral accumulation of acid in the distribution of the superior mesenteric artery. That the responses in the two arteries are potentially the same is evidenced by the cases (as in fig. 2J) in which an administrational and a post-administrational increased flow were seen in the superior mesenteric artery.

It is interesting at this point to observe the effects upon volume flow of destroying the nerve supply to the splanchnic region. In figures 2O, 2P and 2Q are seen the response to administration of 6 and 7 per cent oxygen mixtures. In the experiment represented by figures 2O and 2P the splanchnic nerves and the right vagus were severed. In figure 2Q the first, second and third lumbar ganglia and the splanchnic nerves on each side were removed. In contrast to the effect of low oxygen when the nerve supply was intact, the volume flow is now seen to increase during administration and to decrease during recovery from low oxygen. The similarity to the response to low oxygen of the denervated fore limb and of the brain region is striking.

The effects of sodium cyanide injection after nerve section are seen in figures 2R and 2S. As was the case in the denervated fore limb, section of the nerves supplying the region results in disappearance of the initial decrease in flow.

An interesting variation of the response to low oxygen in the denervated intestinal region is seen in figure 2T. The flow is seen to decrease after a slight temporary rise, a result almost exactly the reverse of the usual one. It will be noted that in this experiment the animal is allowed spontaneous ventilation and that there is a marked hyperpnea. In the other experiments, constant artificial ventilation was used. The most logical explanation of this difference would seem to be that in the experiments in which spontaneous ventilation was allowed, the marked hyperpnea with its coincident washing out of carbon dioxide and its partial compensation for the deficient alveolar oxygen, resulted in an alkaline condition of blood and intestinal tissue, the local effect of alkaline change upon the vessels being constriction (Gesell, Brassfield, Krueger, Nicholson and Pelecovitch 1930). In the experiments with constant ventilation, on the other hand, in the absence of these factors, the tissues and blood tend to turn acid and the direct effects of low oxygen are more pronounced, resulting in relaxation of vessel walls and an increased flow.

As in the case of the femoral and brachial arteries, denervation of the region whose volume flow is being measured verifies the existence of two sets of factors, central nervous and peripheral, and designates upon which of these the observed changes in flow are dependent. Although the experiments do not indicate the mechanisms of the central and peripheral action of low oxygen, the results are not contrary to the existence of dual central

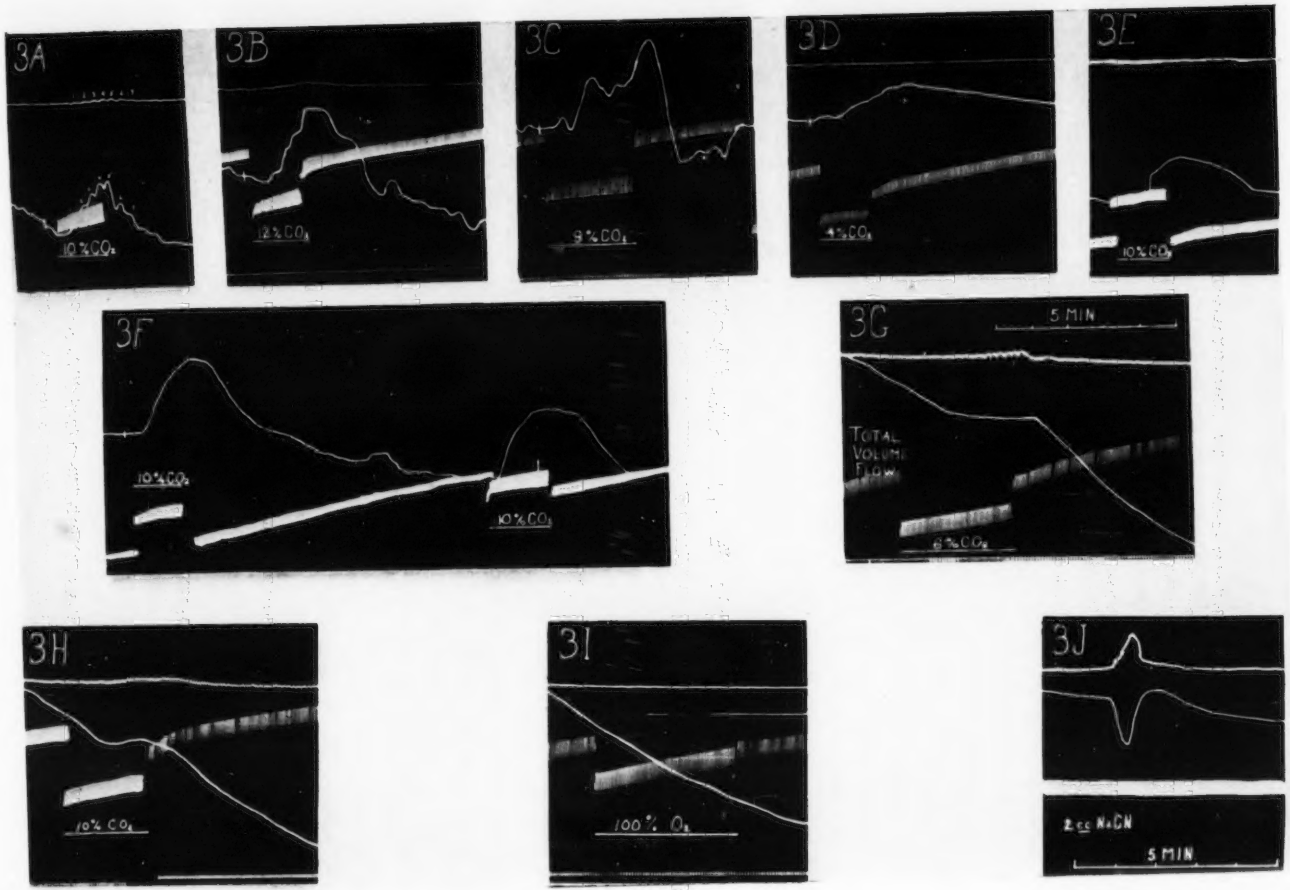
3A
10% CO2
3B
12% CO2
3C
9% CO2
3D
4% CO2
3E
10% CO2
3F
10% CO2
10% CO2
3G
5 MIN
TOTAL VOLUME FLOW
6% CO2
3H
10% CO2
3I
100% O2
3J
2 cc NaCN
5 MIN

Fig. 3

and peripheral mechanisms of volume flow control, both of which may be related to deficient oxidation and to acidity.

2. *The effects of high alveolar oxygen pressures.* The effect of high alveolar oxygen pressures upon volume flow of blood in the superior mesenteric artery are shown in figures 2I and 2U. In direct contrast to the effects of low alveolar oxygen, the flow is seen to rise throughout the period of administration of 100 per cent oxygen and to return to pre-administration levels upon the readministration of room air. In figure 2U the increase in flow is partially offset by a simultaneous fall in the mean blood pressure. In figure 2I taken from the same experiment the blood pressure is kept at a constant level and the increase in blood flow is seen to be more rapid and more pronounced even though the period of administration of the high oxygen mixture is of shorter duration.

In figure 2V taken from another experiment, 100 per cent oxygen is administered after removal of the lumbar ganglia and section of the splanchnic nerves. The response of volume flow is unaltered by the denervation. Whether the mechanism of the action of high oxygen is a purely local one, or whether it involves both central and peripheral effects acting in the same direction is impossible to state upon the basis of these data.

Regardless of what the mechanism may be, it is significant to note that here as in the case of femoral and carotid flows, the effects of low oxygen administration (in innervated tissue) are exactly reversed by the administration of gaseous mixtures high in oxygen contents.

3. *The effects of high alveolar carbon dioxide pressures.* In marked contrast to the results seen in the femoral and brachial arteries, the effect of high alveolar carbon dioxide pressure in the superior mesenteric artery is an increased flow of blood, with recovery upon readministration of room air. These results may be seen in figures 3A, 3B, 3C, 3D and 3E in which 10, 12, 9, 4 and 10 per cent carbon dioxide mixtures, respectively, were administered. In figures 3A, 3B and 3C the blood pressure is allowed to rise as it normally does during carbon dioxide administration, and the increased flow is undoubtedly due in part to this factor. In figures 3D and 3E, however, the mean blood pressure is kept at a constant level during administration of 4 per cent and 10 per cent carbon dioxide mixtures, and an increased flow is seen to occur, nevertheless, indicating that there must be active dilatation of blood vessels. In all of the experiments from which these records were taken, the nerve supply to the distribution of the superior mesenteric artery was intact. Figure 3F is taken from an experiment in which there was complete denervation of the region, and the results shown are typical of all of the experiments in which this was done. Apparently denervation produces no alteration in the response to high alveolar carbon dioxide It is perhaps a significant point in view

Fig. 3

of the similar situation with regard to the flow in brachial and femoral arteries.

When viewed together the effects of high carbon dioxide upon the volume flow of blood in several regions of the body present obvious difficulties of interpretation. With regard to the action of carbon dioxide in animals with nerve supply intact, it has been generally accepted since the work of Mathison (1911) that the increased arterial pressure noted during carbon dioxide administration is a result of vaso-constriction due to stimulation of the vaso-constrictor centers by increased hydrogen ion concentration. The decreased flow in the femoral and brachial arteries is, of course, not contrary to this interpretation. But, if carbon dioxide acts by stimulating vaso-constrictor centers, why does its administration not result in decreased flow in the superior mesenteric artery, the distribution of which is richly supplied with vaso-constrictor fibers? There is the possibility, of course, that a tendency toward decreased flow in the superior mesenteric distribution does exist, but that it is overcome by the local dilating effects of the increased hydrogen ion concentration. However, if this tendency existed, it would be logical to suppose that in some of the experiments at least an initial decrease in flow would precede the increased flow,—a central nervous effect giving way to a more slowly developing local effect. Initial decreased flow upon administration of carbon dioxide has, however, been observed in none of the experiments. Add to these observations the fact that exclusion of central nervous influences by denervation produced no significant alteration in the original response of either femoral or superior mesenteric arterial flow, and it becomes difficult to believe that the predominating effects of carbon dioxide administration upon volume flow are not dependent upon factors other than direct stimulation of vaso-constrictor centers.

Granting for purposes of discussion that the effects of carbon dioxide are not dependent upon impulses passing directly from central nervous system to the vessels, two other possibilities suggest themselves. First, the effects of carbon dioxide may be dependent upon purely local factors, such as a direct action upon the vascular contractile elements. Second, it may by its action in other portions of the body cause to be liberated into the blood stream agencies which are capable of local vasomotor activity.

With regard to the latter possibility some support is given by the work of Itami (1913), who found that the production by carbon dioxide of a decreased limb volume was dependent upon the integrity of the spinal cord from the second dorsal nerve downward even though the nerve supply to the limb itself was destroyed. Apparently carbon dioxide exerted upon the composition of the blood some influence which required the presence of the spinal cord for its production. This influence was

believed to involve the liberation of increased amounts of epinephrine into the blood stream due to impulses coming to the supra-renal glands from the vasomotor center by way of the splanchnic nerves. Doubt is cast upon this interpretation by the work of Kellaway (1919), which indicates that prolonged administration of carbon dioxide in mixtures as high as 20 per cent is without effect upon the epinephrine content of the blood, and by the response to carbon dioxide noted in the flow of the superior mesenteric artery.

With regard to the possibility mentioned above of the effects of carbon dioxide being dependent upon purely local factors, there are again difficulties in interpretation. If carbon dioxide is acting directly upon vascular contractile elements by virtue of increased acidity, or, indeed, by any other mechanism including unknown changes in blood composition, it is logical to suppose that this influence will be generally distributed in the arterial blood stream and that it should therefore affect all of the vascular contractile elements with which the blood comes into contact. Why, then, should there be contraction of these elements in one portion of the body and relaxation in another? Either structures which, so far as we know, are identical are reacting differently to the same stimuli or the characteristics of the contractile elements themselves or the structures (such as nerve endings) governing them are different in the two regions. The first explanation is contrary to accepted biological laws and for the second there is no direct evidence available. Further investigation is obviously necessary before an adequate interpretation of the results can be made. The evidence provided by these experiments seems to indicate, however, that the ultimate determining factor in the vasomotor effects of high carbon dioxide is something other than (or at least in addition to) the vasomotor centers and the contractile elements of the vessel walls. Whether this be differences in the nerve endings or the peripheral ganglia in different types of tissues, differences in metabolic products called forth by carbon dioxide or other unknown factors is speculation only.

Before leaving the subject of superior mesenteric arterial flow a word is necessary concerning the limitations of application of these data. As was stated earlier in the discussion, it was hoped that the flow in the superior mesenteric artery would serve as an index to flow in abdominal viscera in general. There is evidence, however, that its application must be more restricted than this. We may cite as an example one experiment in which the flow in the abdominal aorta (the flow of lower extremities having been excluded) was being measured. Administration of high alveolar carbon dioxide resulted in a marked decrease in flow. The coeliac axis and renal arteries were then occluded and high carbon dioxide again administered, whereupon the response was a marked increase in flow. Apparently there was sufficient vasoconstriction in the distribution

of the coeliac axis and renal arteries to completely mask the usual effect of increased flow in the superior mesenteric distribution.

C. *Total (aortic) volume flow.* In view of the widely varying responses of volume flow of blood in different parts of the body it is of interest to observe what their resultant effect will be upon the total volume flow measured under the same experimental conditions. In figures 3G, H, I and J are shown the effects of each of the experimental conditions outlined upon the volume flow of blood as measured at the arch of the aorta. In experiments of this kind, the circulation deteriorates relatively rapidly, which accounts for the general downward drift. To interpret the results accurately, the basal flow drift must first be visualized and the effects of the experimental procedure superimposed upon it.

Observation of figure 3G indicates that in spite of the initial decreased flow in the femoral and brachial arteries and the decreased flow in the superior mesenteric artery, the effect of six per cent oxygen administration upon the total volume flow is a substantial increase.

Figure 3H indicates that the decreased flow in the muscles, skin, bone, etc., which occurs with high carbon dioxide administration is more than counterbalanced by the increased flow in other regions (superior mesenteric, carotid, etc.) for the total volume flow increased quite markedly with 10 per cent carbon dioxide administration.

Figure 3I indicates that the decreased flow in the limbs and head region upon administration of 100 per cent oxygen is approximately evenly counterbalanced by the increased flow in regions such as that supplied by the superior mesenteric artery. In a limited number of experiments the total volume flow was slightly decreased upon 100 per cent oxygen administration.

Figure 3J indicates that the intense vaso-constriction which occurs in all parts of the body upon the injection of sodium cyanide actually results in a sharply lessened output of the heart in spite of the fact that an enormous increase in arterial blood pressure is effected by the heart. Subsequently the total flow is increased under the influence of sodium cyanide.

SUMMARY AND CONCLUSIONS

The effects of variations in alveolar oxygen and carbon dioxide pressures and of the intravenous injection of sodium cyanide upon the femoral, brachial, superior mesenteric and aortic flow of blood were studied with the continuous electrometric method. Distinction between central nervous and peripheral effects was made so far as possible by comparing the responses in denervated tissues with those in tissues with intact nerve supply.

In the regions supplied by the femoral and brachial arteries, the results with nerve supply intact confirm those described in earlier reports. Low

alveolar oxygen produced an initial decrease in flow of blood which gave way to increased flow above the preadministration level. Readministration of room air resulted in a second rapid and transitory increased flow which eventually gave way to recovery. High alveolar carbon dioxide produced a decreased flow of blood in these regions which was followed by recovery upon readministration of room air. Sodium cyanide caused usually an initial decreased flow which was followed rapidly by an increase far above preadministration levels.

Denervation of the region supplied by femoral or brachial arteries resulted in the disappearance of the initial decreased flow and the post-administrational increased flow which usually accompany the administration of gaseous mixtures low in oxygen. The administrational increased flow was not affected by denervation.

Denervation of the femoral and brachial regions resulted in the disappearance of the initial decreased flow usually seen upon injection of sodium cyanide. The usual increased flow was not affected by denervation.

It is concluded that there are two mechanisms constantly at work in the control of volume flow of blood in this region,—one central nervous and the other peripheral. The results are not opposed to the possibility, previously suggested, of there being a central and a peripheral acid mechanism of volume flow control, modified by the direct action of reduced oxidations, in which the metabolism of the vasomotor centers and of the peripheral contractile elements may play a part.

Attention is called to the resemblance of brain flow (previously described) to the flow in denervated tissue.

The administration of gaseous mixtures high in carbon dioxide decreased the flow in the femoral and brachial arteries even though the nerve suppy to the region was destroyed. Reasons are given for believing that increased viscosity of the blood and increased secretion of the adrenal glands are not factors in producing this effect.

In the superior mesenteric artery the volume flow of blood was decreased by administration of low oxygen mixtures and increased by administration of high carbon dioxide and high oxygen mixtures. Sodium cyanide caused an increased flow usually preceded by an initial decreased flow. These effects occur independently of mean blood pressure changes.

Denervation of the region supplied by the superior mesenteric artery resulted in the main in an increased flow upon the administration of low oxygen mixtures provided constant artificial ventilation was used. If spontaneous changes in ventilation were allowed, the volume flow decreased.

Denervation in the superior mesenteric artery region resulted in absence of the initial decreased flow upon injection of sodium cyanide.

It is concluded that here, as in the femoral and brachial regions, there are two mechanisms,—central and peripheral—involved in the control

of volume flow changes during low oxygen and sodium cyanide administration, and that the results are not contrary to there being an acid mechanism of volume flow control modified by the direct effects of deficient oxidation.

The effects of high carbon dioxide and high oxygen administration upon superior mesenteric arterial flow are not altered by denervation of the region.

Reasons are given for believing that the superior mesenteric arterial flow cannot safely be used as an indication of the flow in abdominal viscera in general.

The resultant of the changes in flow in the several regions of the body upon the total aortic flow was found to be increased flow upon administration of low oxygen and high carbon dioxide, unchanged or decreased total flow upon administration of 100 per cent oxygen, and a markedly decreased total flow followed by an increase upon the injection of sodium cyanide.

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THE INFLUENCE OF RETINAL STIMULI UPON LABYRINTHINE HEAD-NYSTAGMUS OF PIGEONS

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In a series of experiments (as yet unpublished) in which labyrinthine reflexes of pigeons were being studied, visual reflexes and what appeared to be voluntary movements of the head were observed to influence markedly the after-nystagmus and to a lesser extent, the rotation nystagmus. The tracings of the head movements following the cessation of rotation were frequently found difficult to interpret on account of the numerous voluntary movements of the head which were often displayed instead of the typical after-nystagmus. Furthermore, the results obtained from the pigeons used in our series did not coincide with those reported by King (1926) in whose observations on normal squabs, considerable after-nystagmus, ranging from four to twenty-seven nystagmic jerks, appeared in ninety-five per cent or more of the birds examined. Since the squabs used by him were rotated in a well illuminated room, the records of the head-nystagmus obtained by rotation represented the algebraical sum of the visual and labyrinthine reflexes (Loeb, 1907). Observations made by us on a series of twenty pigeons under similar conditions failed to demonstrate an equally frequent occurrence of after-nystagmus in the presence of such visual complications. Less than one-half of the pigeons exhibited typical after-nystagmus following rotation.[2] Many of the tracings in which after-nystagmus might have been considered present were rendered quite indeterminate by numerous atypical movements of the head. In the face of these apparently contradictory results and because of the fact that characteristic labyrinthine after-nystagmus was often masked by the visual responses, it was considered advisable to make further accurate determinations of the optical influence on labyrinthine nystagmus.

METHOD. A motor-drive rotating table similar to the one originally designed by Maxwell (1922) but corresponding more closely to those described by Pilz (1926) and by King (1926) was used in these experiments. The velocity of the rotating table was adjusted so that one revolution was

[1] Assisted by a scholarship from the Henry Strong Denison Medical Research Foundation.

[2] Complete data of only ten pigeons are included in this report.

made every two seconds. The duration of acceleration and deceleration equaled approximately one and one-half turns each. No mechanical break was employed to terminate rotation after the current was shut off, since the friction of the motor, rotating table, etc., was found to be sufficient in itself to produce the desired speed of deceleration. Each pigeon was rotated approximately ten and one-half revolutions.

By means of a simple key, a signal magnet and a chronometer, the duration of rotation in seconds was determined in each experiment. A modified simple key was mounted on the upper surface of the table top and extended over the edge so that the circuit was closed by a stationary contact-maker once each revolution. This arrangement in series with a signal magnet provided for the recording of the number of turns of rotation and at the same time enabled one to count the number of revolutions of the table by the sound produced each time the key was closed.

The pigeon-holder, except for a few modifications, was essentially the same as the one described by King (1926). The head nystagmus was recorded in the following manner: a silk thread, attached by means of an Englemann heart clamp to a small silver ring permanently inset into the beak of the pigeon, was led over a pulley and fastened to a Jackson heart lever which was adjusted to write on a vertically mounted kymograph. The pigeons were first rotated in the light in order to record the combined optical and labyrinthine effects. After a rest period of approximately one hour they were again rotated but this time in complete darkness. Since by this latter procedure the optical influence was completely eliminated, a true representation of the labyrinthine nystagmus was obtained. An accurate determination of the optical influence on head-nystagmus was thus afforded by comparing the records obtained by rotation in the light with those obtained by rotation in the dark. The direction of rotation was reversed on alternate days not only for the purpose of studying the response to rotation in each direction, but also to eliminate as nearly as possible the decrease in nystagmus due to habituation to repeated rotation (Maxwell, 1922; Holsopple, 1923; Pilz, 1926; King, 1926, and others). Daily records were made of the head-nystagmus of both young and adult pigeons in order to determine any difference in nystagmus which might have developed with an increase in age. After pigeons have learned to fly certain modifications of the labyrinthine reflexes might be expected to develop as a result of repeated labyrinthine excitations. It seems conceivable to suppose that with continued stimulation of the labyrinths co-existent with aerial activity, the vestibular mechanism of older pigeons might become more resistant to the changes in the rotational response produced by repeated rotation, and therefore exhibit less habituation. In order to test these suppositions, two groups of pigeons were selected. One group, series I, consisted of five squabs, each approximately three weeks old, and

the other group, series II, was composed of five adult pigeons, each of which was between one and two years of age.

RESULTS. Examples of tracings of the head-nystagmus obtained by rotation in the light and by rotation in the dark are shown in figure 1. The characteristic differences in the nystagmic responses which occur under such conditions are shown even though the examples are records of two different pigeons. It will be noted that both the amplitude and the frequency of the nystagmic jerks of rotation-nystagmus are greater when the optical influence is combined with the labyrinthine response. Exactly similar findings are shown when tracings of the rotation-nystagmus of the same pigeon are compared. Likewise, the post-rotatory responses of the same pigeon produce tracings similar to those illustrated in figure 1. The tracing of pigeon 15, recorded by rotation in darkness, shows marked after-nystagmus following the cessation of rotation, whereas that of pigeon 24, recorded by rotation in the light, displays no after-nystagmus but illustrates instead, a type of complication resulting from voluntary movement of the head. That such irregular, arrhythmic movements are chiefly voluntary movements was ascertained in the following manner. Determinations were made of the post-rotatory head-movement after successively changing the point of termination of rotation, so that the position of the observer with respect to the visual field of the pigeon was correspondingly altered with each change in position. Under such conditions, considerable variation in the amount of head movement was observed in the different positions. For example, when the table was stopped in a position such that the pigeon could not readily see the observer without turning its head, that is, with the head pointing either directly toward or away from the observer, the amount of head movement as a rule was greater than in other positions, the pigeon turning its head first in one direction and then in the other, apparently for the purpose of keeping the observer in view with either one or the other eye. In other positions the amount of head movement varied considerably, but in general was less in those which enabled the pigeon to easily watch the observer with one eye. Thus the position of the observer with respect to the line of vision of the pigeon was found to be a very important factor in determining the extent of movement of the head following rotation in the light, and for this reason, these movements are believed to be voluntary in nature. Individual pigeons of course showed considerable variation in the amount of post-rotatory voluntary head-movement, some showing only a few atypical head-jerks or no movement at all, while others displayed extensive head movement such as that shown by pigeon 24.

Voluntary movement of the head is no doubt only one of several factors which interfere with the exhibition of labyrinthine after-nystagmus. Loeb (1907) showed that retinal stimuli following rotation produced nystagmic

effects which were exactly opposite in direction to those resulting from labyrinthine excitation, and that the extent of after-nystagmus displayed by an animal when the two influences were acting simultaneously, is the algebraical sum of the positive labyrinthine factor and the negative retinal factor. In addition to this, numerous investigators have shown that, with

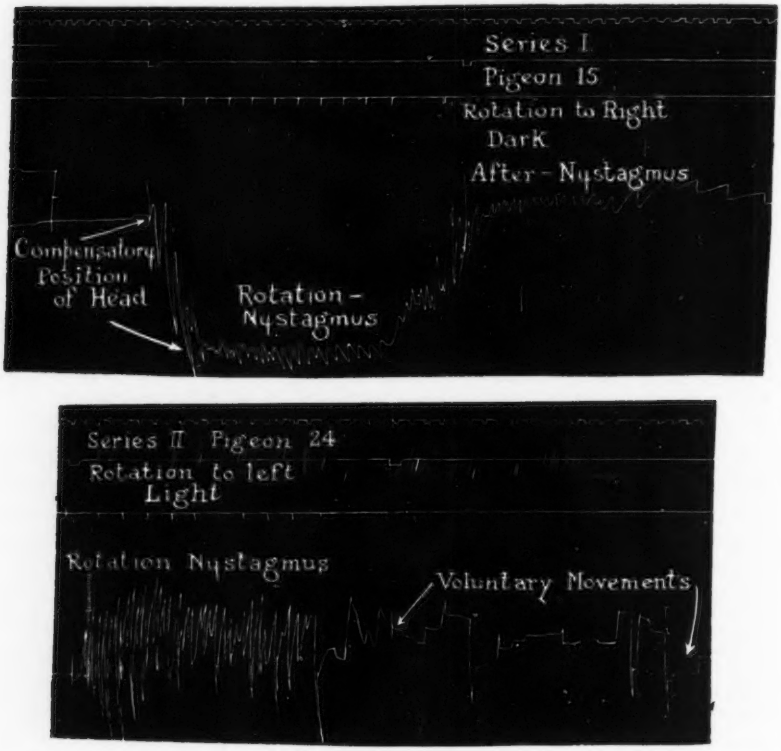

Fig. 1. Showing examples of tracings of the head-nystagmus of pigeons produced by rotation in the light and by rotation in the dark. Each record shows from above downward; 1, time in seconds; 2, the beginning and the end of rotation; 3, the number of revolutions of the table; and 4, the tracings of the head-movement during and following rotation.

proper excitation of the retinal receptors, an optical nystagmus is elicited which is reflex in nature and very similar to labyrinthine nystagmus. It is believed therefore that in the absence of retinal stimuli, voluntary head movement would be greatly reduced or not present at all. This assertion is borne out in each of the tracings of every pigeon examined by rotation

in darkness; when retinal stimuli were excluded a considerable amount of after-nystagmus was recorded invariably, and none of the records showed any irregularities in either the frequency or the amplitude of the nystagmic jerks which might suggest voluntary movement of the head.

A comparison of the rotation-nystagmus of pigeons 15 and 24 illustrates an interesting difference in the type of rotational response which was observed in several of the birds of both series. The nystagmic jerks of the former are small and are superimposed upon a compensatory deviation of the head which is of relatively large magnitude, whereas those of the latter are of large amplitude and are accompanied by a relatively small amount of compensatory deviation of the head. This reciprocal relation between the amplitude of the nystagmic jerks and the magnitude of the compensatory head position exemplifies the normal physiological variation in the response of individual pigeons to rotation.

All tracings of the pigeons of both series were carefully analyzed and the results compiled in both tabular and graphic form in order to determine quantitatively the influence of retinal stimulation on labyrinthine nystagmus. It was found that in both series the average number of nystagmic jerks per 10 seconds during rotation-nystagmus is always greater when the pigeons are rotated in the light than when they are rotated in the dark. An estimation of the additional rotation-nystagmus showed an increase in frequency which ranged from 18 to 92 per cent. Only slight differences in the rotation-nystagmus were found to exist between squabs and adult pigeons. In contradistinction to the findings observed in rotation-nystagmus, the results of after-nystagmus showed the average number of nystagmic-jerks to be several times greater following rotation in the dark. The average increase in after-nystagmus which occurred when retinal stimuli were excluded was found to be 385 per cent in series I (squabs) and 443 per cent in series II (adult pigeons). It is apparent that the average amount of after-nystagmus shown by adult birds is considerably greater than that shown by squabs. An explanation of this difference in response to repeated rotation exhibited by young and adult pigeons was afforded by a table of results which represented the data obtained from individual records. The decline in the frequency of the after-nystagmus was found to be much more rapid in squabs than in adults. Thus the more rapid habituation to repeated rotation is sufficient to explain why the average number of nystagmic jerks of after-nystagmus of squabs is less than that of adults. Other data from the same table showed: 1, that the average duration of after-nystagmus in seconds is considerably greater in adult pigeons than in squabs, and 2, that, following rotation in the dark, the duration of after-nystagmus is from 2 to 10 times greater than that following rotation in the light.

DISCUSSION AND CONCLUSIONS. The optical influence on the head-nystagmus of pigeons was first observed by Ewald (1892), who found that the rotation-nystagmus was decreased and the after-nystagmus was increased when the retinal stimuli were excluded by placing a uniformly gray cylinder around the pigeon during a rotation experiment. More accurate quantitative determinations on the relationship between visual and labyrinthine responses have been reported by King (1926), who compared the responses of a series of squabs before and after blinding them, and found that the average reduction in the number of nystagmic jerks during rotation nystagmus equaled 59 per cent, and that the number of after-nystagmic jerks increased 97 per cent. From a qualitative standpoint these results are similar to our findings, namely, that retinal stimulation increases the rotation-nystagmus and diminishes or inhibits the after-nystagmus. When compared quantitatively, however, the results show certain differences. Ninety-five per cent or more of the pigeons used by him showed typical after-nystagmus whereas in our series less than 50 per cent of the birds showed typical after-nystagmus. The percentage of reduction in the frequency of rotation-nystagmus obtained by him following the elimination of the optical influence is quite comparable to that found by us in series I, namely, a 59 per cent reduction as compared to a 52 per cent reduction. When the after-nystagmus is compared, however, marked differences are to be seen; he found an increase of 97 per cent in the number of head movements during after-nystagmus in blind pigeons as compared to an increase of 385 per cent observed by us in series I, following rotation in darkness. Whether the different methods employed caused the differences in the rotational response or whether they were caused by fundamental constitutional differences of the pigeons used in the two instances we are not prepared to say. At any rate, it is significant to note that retinal stimuli profoundly modify the labyrinthine response following rotation, and that in any investigation dealing with the study of labyrinthine function, it is important to exclude the complications which arise from visual impulses. These undesirable complications are very readily avoided by rotating the animals in a dark-room. This method of excluding retinal stimulation has a further advantage in that it is unnecessary to cause any injury to the animals which might alter the physiological balance of the central nervous system.

As previously mentioned, the labyrinthine responses of squabs and of adult pigeons to repeated rotation do not coincide. In adult pigeons there is less habituation to repeated rotation in both rotation-nystagmus and after-nystagmus, but especially in the latter. The optical influence on the labryinthine head-nystagmus during rotation also appears to be less pronounced in old pigeons than in squabs, the average number of nystagmic

jerks being increased 45 per cent by the additional retinal stimulation in the former as compared to an increase of 52 per cent in the latter. From these results it would appear that adult pigeons should be the birds of choice in experiments which deal with repeated rotation.

SUMMARY

1. The graphic method of recording head-nystagmus employed in these experiments is described.

2. The procedure followed in the study of the retinal influence on head-nystagmus consisted in recording first the combined effects of retinal and labyrinthine reflexes resulting from rotation in the light, after which a second tracing was recorded one hour later, but this time in complete darkness so that the labyrinthine nystagmus alone could be determined. A comparison of the two records thus obtained afforded an accurate means of measuring the optical influence on the nystagmus of each pigeon.

3. Less than 50 per cent of the pigeons examined showed typical after-nystagmus when rotated in the light but when rotated in the dark it was invariably displayed by every bird.

4. The after-nystagmus of most pigeons following rotation in the light was found to be either completely or incompletely inhibited by visual reflexes in combination with voluntary movements of the head.

5. Both the frequency and the amplitude of the nystagmic jerks were found to be appreciably increased by retinal stimuli during rotation nystagmus.

6. During rotation nystagmus the normal physiological variation of individual pigeons in response to rotation is exemplified by a reciprocal relation between the amplitude of the nystagmic jerks and a relatively fixed compensatory deviation of the head.

7. Adult pigeons are less susceptible to habituation to repeated rotation than squabs. For this reason the former are considered to be more suitable than the latter for experiments dealing with repeated rotation.

8. The results of this investigation indicate that retinal stimuli profoundly modify labyrinthine nystagmus and therefore should be properly excluded in experiments which deal with the study of labyrinthine nystagmus. Such visual complications are very readily eliminated by recording the nystagmus in a dark-room.

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THE THERMIC EFFECT OF DEATH AND HEMOLYSIS

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In my former publication concerning the thermic effect of death (1), I came to the conclusion that death, observed on yeast cells, is accompanied by the production of heat, which is about 2 calories per gram of dry substance of the cells and is therefore very small as compared with the heat produced in most exotherm chemical reactions. The molecular heat produced by the reaction which brings about death is, however, much greater than the molecular heat produced by the decomposition of the most unstable substances (explosives). This conclusion explained the instability of the principal chemical compounds composing living matter.

In the above publication I mentioned Meyerhof's paper in which the author described his experiments on the poisoning of erythrocytes by acroleine. He did not find any distinct production of heat in this process, though the erythrocytes showed no respiration. This observation caused me to continue my experiments upon the thermic effect of death, using erythrocytes.

Before I proceed to describe these experiments, I should like to remind the reader that erythrocytes may be killed by a coagulation of their protoplasm (fixation) under the influence of some poisons or heat. The same poisons taken in weaker concentration and several other harmful factors produce, however, hemolysis. It is evident that this process must also be considered as a kind of death of erythrocytes.

In my investigations on the thermic effect of death, I have therefore to determine the production of heat in both processes: in the fixation and in the hemolysis of red blood corpuscles.

MATERIAL AND METHOD. In my present investigations on the thermic effect of death I used the same apparatus as before, but some improvements were made to obtain more exact results. The Dewar flask used as a calorimeter was larger than in my former experiments (its volume being 650); it was closed by a rubber stopper with three openings instead of two used in my former experiments. Through the first opening a Beckmann thermometer was inserted; in the second opening a short glass tube was fastened on the upper end of which a rubber tube provided with a rubber

ball and a pinchcock was put on. The lower end of the same tube was provided with a 4-inch-long rubber tube, which was connected with the glass vessel containing a solution of poisons. This vessel (or tube) was described in my former publication where also its picture was given (fig. 1, p. 347). In the present experiments I used two larger vessels of this kind. One of these vessels had a volume of $6\frac{3}{4}$ cc. (diam., 1.3 cm., length 7 cm.), the other, 12 cc. (diam., 1.8 cm., length 7 cm.). The volume of the liquid in the Dewar flask was correspondingly either 400 cc. or 600 cc. When the Dewar flask was to be closed with the rubber stopper in which the Beckmann thermometer and the tubes connected with the vessel filled with a solution of poison were inserted, care was taken that all the mercury of the thermometer was immersed in the liquid in the flask. At the same time, it was very important that the vessel with poison lay on the bottom of the flask, immersed in the liquid. The third opening in the rubber stopper was provided with a thin glass tube carrying a rubber tube and a pinchcock. The latter was closed during the experiment and was opened only when the poison was to be blown out into the liquid by means of the above rubber ball after the pinchcock was opened. When the poison had been blown out, both pinchcocks were closed again. The Dewar flask was immersed in a water bath of the same temperature as that of the liquid in the flask. In order to make the temperature of the poison exactly equal to this temperature, the vessel with the poison remained in the flask for three hours (in my former paper it remained for one hour). Other details about the apparatus and the whole procedure of the determination of the thermic effect of death may be found in my former publication.

The red corpuscles used in my experiments were obtained from defibrinized cow blood. Separated from blood serum by centrifugation, they were washed with a 1 per cent solution of sodium chloride three times, and then again centrifuged in every experiment for 20 minutes (2700 revolutions in one minute), and their volume was determined. This volume was mostly about 100 cc. Then the corpuscles were mixed with a 50 times greater volume of a 1 per cent solution of sodium chloride, and the suspension (2 per cent) was left in an ice-box at 0°C. for 15 hours. The salt solution was then poured away from the settled corpuscles by a siphon, and the rest of the suspension was diluted with the salt solution again to the volume of 800 or 1200 cc. Then the liquid was shaken and divided into two exactly equal parts (the volume of each 400 or 600 cc.). This procedure was necessary to completely separate the corpuscles from blood proteins, and to obtain two suspensions of the same volume and containing the same amount of corpuscles. This was necessary because the preliminary experiments showed that the chemical action of poisons on hemoglobin produces more heat than this action on yeast proteins,

hemoglobin having a structure different from nucleoproteids and proteins. Thus only the comparison of the thermic effects produced by the action of poisons upon living and dead corpuscles permits us to exactly determine the thermic effect of death in this case.

The death and hemolysis of red corpuscles were produced in my experiments by chloroform and corrosive sublimate. These substances were chemically pure and received from Merck.

In order to produce the death of red corpuscles the above vessels were filled with saturated solutions of corrosive sublimate, or with chloroform. But even this amount of poisons was not enough to bring about the death of all corpuscles. The use of larger vessels for poisons was, however, impossible because of the size of the Dewar flask.

In order to determine the amount of dead corpuscles in the solution which had been treated by a poison, I used a colorimetric method. I diluted a part of the solution with a 1 per cent solution of sodium chloride until the concentration of corpuscles became 1 cc. in 100 cc. of the solution; then I separated the liquid from the corpuscles by centrifugation, and compared its color with the color of differently concentrated solutions of red corpuscles in water. In this manner I could determine the amount of the corpuscles hemolysed after the action of the poison. This amount is expressed below in percentage of the whole amount of corpuscles treated by the poison. Another part of the solution I diluted with water until the concentration of the corpuscles became 1 cc. in 100 cc. of the solution, then separated the congealed (fixed) corpuscles by centrifugation, and compared the color of the liquid with the color of the solutions of the corpuscles in water. In this way I could determine the percentage of living corpuscles because the congealed cells do not dissolve in water. It should be mentioned that sometimes a weak hemolysis was observed before the action of poisons; this must be considered in the determination of the amount of dead corpuscles.

The thermic effect is expressed below, as usual, in calories per one gram of dry substance of corpuscles. The specific weight of the corpuscles which had been centrifuged for 20 minutes was found to be 1.075, and their water content 69 per cent. The amount of calories per 1 cc. of blood corpuscles is therefore every time divided by 0.33 to express the thermic effect in calories per gram of dry substance. It is evident that only the dry substance of the corpuscles which died after the action of the poisons must be considered.

In my previous experiments on the thermic effect of death, determined on yeast cells, the respiratory processes could not be observed in the solutions containing no sugar. I am not certain whether the respiration of red corpuscles ceased in the solution of sodium chloride after their separation from blood substances, but the heat produced in the respiration

under such conditions is surely very small. Indeed, in my experiments I have never observed an increase of temperature in the Dewar flask greater than 0.018°C. during three hours, and sometimes a decrease of temperature was distinctly observed. The temperature rise produced by the respiration during the five minutes lasting action of poisons upon the corpuscles can be therefore disregarded.

THE HEAT CAPACITY OF THE DEWAR FLASK AND THE BECKMANN THERMOMETER, AND THE SPECIFIC HEAT OF RED CORPUSCLES. In my former publication the volume of the Dewar flask used was 400 cc., and its heat capacity (that is, the amount of calories necessary to raise its temperature by 1°C.) was found to be 15. The volume of the flask used in the present experiments was 600 cc., and its heat capacity was found to be 23.2 (as an average from 24.4, 21.9, and 23.4).

The Beckmann thermometer used in my experiments contained about 30 grams mercury, and its heat capacity was therefore about 0.9.

The specific heat of red corpuscles was determined in the following manner, considering that the specific heat of a 1 per cent solution of sodium chloride is 0.992.

One hundred one and two-tenths grams of blood corpuscles at the temperature of 13.9°C. were mixed in the Dewar flask with 99.7 grams of a 1 per cent solution of sodium chloride at the temperature of 38.2°C. The corpuscles were poured into the flask before the solution of sodium chloride. The temperature of the mixture was found to be 26.0°C. Therefore:

$$101.2\,x\,(26 - 13.9) + 23.3\,(26 - 13.9) = (38.2 - 26) \times 99.7 \times 0.992$$

where x is the specific heat of the corpuscles. It is equal to 0.753.

This specific heat was found in two other experiments to be 0.736 and 0.748. The average heat is therefore 0.746. For a comparison it may be reminded that Rubner found the specific heat of yeast cells to be 0.785.

THE THERMIC EFFECT OF DEATH AND HEMOLYSIS PRODUCED BY CHLOROFORM. Ninety cubic centimeters of red corpuscles treated as indicated above were mixed with 710 cc. of a 1 per cent solution of sodium chloride, and the suspension was divided into two equal parts. One part was poured into the Dewar flask, and the above glass vessel containing 10 grams chloroform was introduced at once into the flask. The water in the bath in which the flask was immersed and the liquid in the flask had the temperature of 20.7°C. After the flask had been closed with the stopper the Beckmann thermometer showed the temperature of 2.907°. After three hours, this temperature was 2.917°. After the liquid had been stirred (by shaking the flask) several times the final temperature was 2.925°, and it did not change more after the further shaking of the flask. Immediately the chloroform was blown out of the glass vessel into the suspension of cor-

puscles, and the liquid again stirred. The temperature of the liquid increased at once to 2.952°. The colorimetric method showed that chloroform produced a hemolysis of the corpuscles, but only 15 per cent of the cells were hemolysed, the rest of the corpuscles remaining alive.

The heat produced by this hemolysis increased the temperature of 355 cc. of the 1 per cent solution of sodium chloride, of 45 cc. of corpuscles, of 10 grams chloroform, of the flask and of the Beckmann thermometer by 0.027°C. This heat is therefore equal to:

$$0.027\ (355 \times 0.992 + 45 \times 1.075 \times 0.746 + 10 \times 0.23 + 23.2 + 30 \times 0.03) = 414 \times 0.027 = 11.2 \text{ gram calories}$$

This heat was produced partly by the action of chloroform on hemoglobin and other substances composing the red corpuscles. In order to determine the heat produced by this action I killed the corpuscles by high temperature. I heated the second part of the above suspension containing also 45 cc. of corpuscles to 80°C., and then determined the heat produced by chloroform in the same manner as described before. After 10 grams of chloroform were blown out into the Dewar flask, the temperature rose at once from 2.497° to 2.507°. The heat produced was therefore: $0.01 \times 414 = 4.1$ calories. The difference of $11.2 - 4.1 = 7.1$ calories could be considered as the heat produced by death. My experiments showed however that this heat is too great in our case, that is in the case of hemolysis.

Indeed, chloroform seems to form a chemical compound with unchanged hemoglobin (2), and this formation is probably accompanied by the production of heat, while chloroform does not apparently form any compound with hemoglobin changed chemically by heating to 80°C. (at this temperature a denaturation takes place). We may therefore expect that the heat produced by the action of chloroform upon the unchanged hemoglobin of living corpuscles is greater than the heat produced by its action upon the corpuscles killed by heating.

In order to determine the heat produced by the action of chloroform upon unchanged hemoglobin, I dissolved 45 cc. of red corpuscles in 555 cc. of distilled water and left the solution for three days at 0°C. Then I determined the heat produced by the action of chloroform on this solution in the Dewar flask. After 10 grams of chloroform were blown out into the solution, its temperature increased at once from 3.482 to 3.492. The heat produced was therefore equal to $0.01 \times 613 = 6.2$ calories. This heat is greater than the heat produced by the action of chloroform on the corpuscles killed by heating.

Thus the heat produced by the hemolysis itself may be estimated as $11.2 - 6.2 = 5.0$ calories. Considering that only 15 per cent of the corpuscles were hemolysed in the first experiment we come to the conclusion

that the thermic effect of death is $\frac{5.0}{6.7 \times 0.33} = 2.2$ calories per one gram of dry substance.

THE THERMIC EFFECT OF DEATH PRODUCED BY CORROSIVE SUBLIMATE. One hundred twenty cubic centimeters of red corpuscles separated from blood proteins were mixed with 680 cc. of a 1 per cent solution of sodium chloride, and the suspension was divided into two equal parts. One part, containing 60 cc. of corpuscles, was poured into the Dewar flask, and at once the above vessel containing 12.5 cc. of a saturated solution of corrosive sublimate was introduced into the flask. Both had the temperature of 20.7°C. After the flask had been closed with the stopper the Beckmann thermometer showed the temperature 2.161°. During the next three hours this temperature increased to 2.177°. After the liquid was stirred by shaking the flask several times, the final temperature was 2.187°, and it did not change more after the further shaking of the flask. Immediately the solution of sublimate was blown out of the glass vessel into the suspension of corpuscles, and the liquid again stirred. The temperature of the liquid increased at once to 2.252°. The colorimetric method showed that sublimate congealed only 20 per cent of the cells. The rest of corpuscles remained alive. This may be explained by the fact that sublimate could not be discovered in the liquid, and was presumably absorbed by dead corpuscles.

The heat produced by the coagulation (fixation) of the corpuscles by corrosive sublimate increased therefore the temperature of 340 cc. of 1 per cent solution of sodium chloride, of 60 cc. of corpuscles, of 12.5 cc. of the solution of corrosive sublimate, of the Dewar flask and of the Beckmann thermometer by 0.065°C. This heat is equal to:

$$0.065\,(340 \times 0.992 + 60 \times 1.075 \times 0.746 + 12.5 \times 1.002 + 23 + 0.9) = 421 \times 0.065 = 27.3 \text{ calories.}$$

From this heat, the heat produced in the action of corrosive sublimate on hemoglobin and other substances composing the corpuscles must be subtracted. This heat was determined in a similar manner as in the case of the action of chloroform. The second part of the same suspension of corpuscles (containing 60 cc. of corpuscles) was preliminary heated to 80°C. so that the corpuscles were congealed, and then the corrosive sublimate was mixed with this suspension in the Dewar flask. The temperature rise produced by corrosive sublimate was estimated as 0.043°C. The heat produced was therefore $421 \times 0.043 = 18.1$ calories. Thus, the thermic effect of death itself is $27.3 - 18.1 = 9.2$ calories, or per gram of the dry substance of dead corpuscles, $\frac{9.2}{12 \times 0.33} = 2.3$ gram calories.

Although corrosive sublimate as well as heating brings about a coagulation of the protoplasm of red corpsucles, the color of the corpuscles killed by sublimate is red, and only gradually becomes brownish, while they become brownish at once if heated to 80°C. This shows that hemoglobin is changed more by heat than by sublimate. One could think therefore that if the corpuscles had been killed without a change of color the action of sublimate on them would produce more heat than in the case of killing them by heating. My experiments showed, however, that in both cases the thermic effect of death is approximately the same, as it is seen from the following.

Ninty-eight cubic centimeters of red corpuscles were treated as before and dispersed in 500 cc. of a 1 per cent solution of sodium chloride. The suspension was divided in two equal parts, one of which was diluted with the same salt solution to the volume of 400 cc. and submitted in living state to the action of corrosive sublimate. To the other part of the suspension 12 cc. of a 2 per cent solution of saponin were added and the mixture was left for 10 days at 0°C. After this time all corpuscles were hemolysed. The hemolysed suspension of the corpuscles was diluted with the same salt solution to the volume of 400 cc. and then submitted to the action of sublimate. The temperature increase produced by mixing this poison with the first (living) suspension was 0.061°C., while that in the second case was 0.041°C. The corresponding production of heat was therefore $424 \times 0.061 = 25.8$ and $424 \times 0.041 = 17.4$ calories. The difference of 8.4 calories was produced by death itself. As the colorimetric method showed that only 25 per cent of the corpuscles were killed (fixed) by corrosive sublimate, the thermic effect of death per one gram of dry substance is $\frac{8.4}{12 \times 0.33} = 2.1$ calories.

SUMMARY

The experiments made on red blood corpuscles confirmed the author's former experiments on the thermic effect of death, determined on yeast cells. The hemolysis or the coagulation of protoplasm of red corpuscles is accompanied by the production of heat to the amount of 2.1 to 2.4 calories per one gram of dry substance of corpuscles. The death of red corpuscles as well as that of yeast cells is therefore an exotherm process. This confirms my supposition that death is accompanied by the decomposition of some very unstable chemical compounds. In the case of red corpuscles, they are probably the compounds of hemoglobin with sterins and phosphatids (3). As the molecular weight of hemoglobin is not less than 16,600, the decomposition of the molecule of its unstable chemical compounds forming the protoplasm of red corpuscles is accompanied by

the production of at least $2 \times 16{,}600 = 33{,}200$ calories. This thermic effect is greater than that produced by the destruction of the molecule of some explosive substances.

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ANATOMICAL AND BEHAVIOR CHANGES PRODUCED IN THE RAT BY COMPLETE AND PARTIAL EXTIRPATION OF THE PITUITARY GLAND

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It is now a well-established fact that spontaneous activity of an organism is markedly dependent on the function of the endocrine glands. Wang (1923) and Hoskins (1925) showed that in an adult rat spaying or castration produces a large, permanent decrease (65 to 95 per cent) in spontaneous activity as measured by the amount of running in a revolving drum. However, when the sex glands are removed before puberty the animals remain inactive throughout life. It was found further that a large decrease in spontaneous activity may be produced merely by functional inhibition of the glands. Thus, the inhibition of the ovaries occurring during pregnancy and pseudo-pregnancy is associated with a very large decrease in spontaneous activity. These observations establish clearly the fact of the dependence of spontaneous activity on the sex glands. That the sex glands are not exclusively responsible for the production of spontaneous activity has been brought out by the observation that removal of the adrenal glands also is followed by a large decrease in activity. This result indicates that spontaneous activity may be dependent on the interrelationship between several glands rather than on any one gland. In order to throw further light on the problem we have undertaken to study the effects produced by extirpation of other glands, in the present instance the pituitary gland.

In addition to studying the effect produced on spontaneous activity by extirpation of the pituitary gland, observations were made simultaneously upon food and water-intake, on body-weight, and sex activity. Subsequently each animal was autopsied to determine just how much of the gland had been removed and to what extent the different organs of the body were affected by the hypophysectomy.

METHOD. The technique of removing the gland was the same as that used by Smith (1927) except for a few modifications, some of which have been described in another paper (Richter, 1930). It is necessary to mention only a few of the details. The anesthetized animal is tied on its

TABLE 1

The first column gives the identification number; the second, the sex; the third, the age at the time of operation; the fourth, the duration of the experimental period up to the time the animals were killed; the fifth column gives in brief the autopsy finding; the sixth, a record of the anterior lobe injections. The average daily running activity for the last twenty days of the experiment and the highest peak reached at any time are given in the seventh and eighth columns; changes in body-weight and food-intake appear in the ninth and tenth columns; the results of the vaginal smear examinations are recorded in the eleventh column. In the last two columns the average daily water-intake for the entire experimental period and the highest peak reached are presented.

RAT NUMBER	SEX	AGE	EXPERIMENTAL PERIOD	PITUITARY	INJECTION PERIOD	AVERAGE DAILY ACTIVITY IN REVOLUTIONS	HIGHEST PEAK ACTIVITY	BODY WEIGHT	FOOD INTAKE	SEX ACTIVITY	WATER-INTAKE	
											Average	Highest peak
		days	*days*		*days*						*cc.*	*cc.*
577	♀	194	64	Compl. removal	31	315	700	Decr.	Decr.		22	46
595	♂	202	60	Compl. removal	32	762	1,900	Decr.	Decr.		44	74
615	♀	170	64	Compl. removal		72	800	Decr.	Decr.		13	158
640	♂	76	53	Compl. removal	31	168	400	Decr.	Decr.	Very low	28	85
641	♂	75	52	Compl. removal	33	131	600	Decr.	Decr.		22	47
644	♂	57	56	Compl. removal	31	44	150	Decr.	Decr.		26	43
645	♀	160	50	Compl. removal		220	500	Sl. decr.	Decr.		26	70
647	♀	145	52	Compl. removal	31	75	150	Decr.	Decr.		23	82
648	♂	52	51	Compl. removal	31	367	2,500	Decr.	Decr.		23	40
652	♂	66	44	Compl. removal	31	6	200	Decr.	Decr.		21	42
Average						216	790				25	69

597	♀	203	44	Deg. ant. lobe		22	100	Decr.	Decr.	Irr. 4 day cycle	41	86
584	♂	199	65	Portion of ant. lobe		38	179	Decr.	Decr.		35	110
588	♀	200	65	Portion of ant. lobe		6,443	23,000	No ch.	No ch.	4 day	48	105
601	♀	182	73	Portion of ant. lobe		3,950	9,300	No ch.	No ch.	4 day	56	89
602	♀	183	72	Portion of ant. lobe		7,760	14,900	No ch.	No ch.	4 day	53	101
607	♀	186	69	Portion of ant. lobe		1,498	13,700	No ch.	No ch.	4 day	48	99
620	♀	170	66	Portion of ant. lobe		1,693	5,700	Incr.	No ch.	4 day	28	40
634	♂	112	59	Portion of ant. lobe		160	600	Incr.	No ch.		31	90
636	♂	114	53	Portion of ant. lobe		851	2,100	Incr.	No ch.		47	142
637	♂	110	56	Portion of ant. lobe		2,394	2,800	Incr.	No ch.		36	50
574	♀	177	88	Portion of ant. and post. lobe		7,364	10,100	Incr.	No ch.	Cont. corn. cells	23	45
600	♀	184	73	Portion of ant. and post. lobe	30	1,728	5,900	Decr.	Decr.	No corn. cells	17	30
608	♀	186	69	Portion of ant. and post. lobe		1,167	10,900	Incr.	No ch.	4 day	20	67
609	♀	166	86	Portion of ant. and post. lobe		28	9,800	No ch.	No ch.	Irr. cycle	16	35
638	♂	80	43	Portion of ant. and post. lobe		5,603	8,500	Incr.	No ch.		45	84
Average						2,713	7,839				36	78

back and a longitudinal incision is made slightly to one side of the midline through the muscles of the neck and pharynx. With two probes the muscles can be easily separated thus exposing the bone at the base of the skull. At this stage Smith makes an opening through the nasal cavity and scrapes away the membrane overlying the bone. We have found that the operation may be somewhat simplified if the nasal cavity is left intact and the bone is exposed instead by separating the membranes from behind the cavity. The desired point of entrance on the skull can be exactly located by means of the occipito-transverse suture. The opening is made with a dental burr rather than a trephine as is used by Smith. A small hole is made in the midline 1 mm. anterior to the suture. Thereupon the hole is widened with a larger burr. The membranes covering the gland are then ruptured and the gland is sucked out through a glass cannula made especially for this purpose. The optical and lighting systems worked out by Smith make it possible to see clearly at all times just what is being done in the operative field.

As in previous experiments (Richter, 1927) the rats were kept in separate activity cages containing food-box, watering tube and a small living compartment, besides the revolving drum. Daily readings were made of the food and water-intake and of the spontaneous activity as recorded on the cyclometer. Vaginal smears were taken daily and the animals were weighed once each week.

Twenty-five rats varying in age from 52 to 202 days were used in these experiments. After the operation they were kept alive for 43 to 88 days before they were killed and autopsied.

Ten of the animals received daily injections of alkaline extract of the anterior lobe of sheep, in order to determine to what extent this would correct the defects produced by hypophysectomy, especially the diminished spontaneous activity.

Results. The results of these experiments are presented in table 1. The animals are listed in two groups: the first group contains the animals in which the pituitary was totally removed; the second, those in which it was only partially removed. This latter group is further subdivided into those animals with only a portion of the anterior lobe remaining and those in which portions of both anterior and posterior lobes were left.

The table shows that in ten out of the twenty-five animals the hypophysis was completely removed, while in fifteen it was only partially removed. In ten of these animals only anterior lobe was present; in five, remnants of both anterior and posterior lobes were found.

At autopsy the ten animals of the "total removal" group showed a very constant and characteristic picture. In keeping with Smith's observations they showed a very marked atrophy of the adrenals, thyroid and sex glands. In addition, however, they showed an hypertrophy of the lym-

phoid tissues, especially the thymus gland. These changes were absent in all excepting three of the "partial removal" group.

Spontaneous activity. Removal of the pituitary gland has a markedly depressive effect on spontaneous activity as measured by the amount of running in the revolving drum. After complete extirpation of the hypophysis the animals become almost totally inactive. Figure 1 shows the running records of two animals, one in which the gland was only partially removed, and the other after a total hypophysectomy. The partial

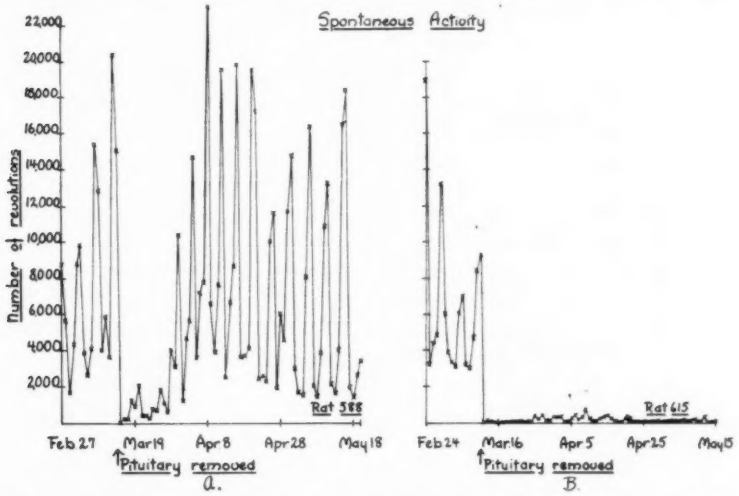

Fig. 1. Graphs showing the effect on activity of partial and total removal of the pituitary gland. Spontaneous activity as measured by the number of turns of the revolving drum is indicated on the ordinates and the duration of the experiment in days on the abscissae. *A* shows the graph from rat 588 in which a small part of the anterior lobe was still present. It can be seen that the activity level was unaffected except for a few days following the operation. The four day cycles are present. *B* shows the graph for rat 615 in which the gland was entirely removed. There is a marked permanent drop of activity to a low level and the cycles are entirely absent.

removal has no effect on activity, while the total removal produces an immediate and permanent drop in activity. Table 1 shows that the running average for the ten animals with total removal of the gland was 216 revolutions as compared to 2713 revolutions for the group in which the gland had been partially removed. The average highest peak reached was 790 after total extirpation and 7839 revolutions after partial extirpation.

The results show that a low level of activity is present after removal of the pituitary just as it is after ablation of the sex glands or the adrenals. In view of this fact and the further observation that the adrenals and sex

glands become markedly atrophied after pituitary extirpation, the low level of activity of the hypophysectomized rat might be interpreted as being dependent in the last instance upon the altered activity of these glands rather than on the removal of the pituitary itself. At any rate, these findings indicate definitely that activity is controlled by the interplay of a number of the ductless glands.

A similar condition of inactivity after hypophysectomy has been observed by Sato (1928) in dogs. This observer found that his hypophysectomized dogs lived and thrived, but that they were very apathetic and sluggish.

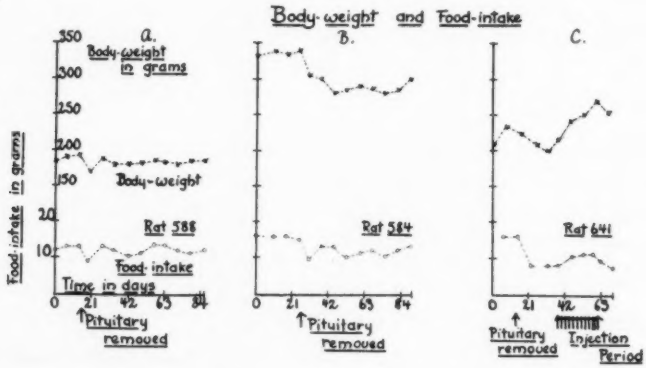

Fig. 2. Graphs showing the effect produced on body-weight and food-intake by hypophysectomy. Body-weight in grams and food-intake in grams are shown on the ordinates, and the duration of the experiment in days on the abscissae. *A* is a graph of a partially hypophysectomized rat in which any effects on either body-weight or food-intake are lacking, except for the few days immediately following the operation. *B* shows a typical graph of a totally hypophysectomized animal in which the sudden and permanent drop in both body-weight and food-intake can be seen. *C* shows the effect of injection of alkaline anterior lobe extract on body-weight and food-intake.

Body-weight. In keeping with the observations of Smith it was found that hypophysectomy produces a decrease in body-weight. The decrease is, of course, much more marked in young than in older animals. Nine out of ten of the "total removal" group showed a definite decrease; in one animal the decrease was very slight. The absence of any effect on body-weight in a partially hyphysectomized rat is shown in figure 2 *a*, and the sudden and marked effect on body-weight in a totally hypophysectomized rat is shown in figure 2 *b*. It is interesting to note that in most of the animals body-weight does not continue to decrease, but after a rather sharp initial drop within the first two weeks, reaches a low but fairly permanent level. Three of the fifteen partial removal group showed a decrease. In

one of these the anterior lobe cells were in a degenerated condition. In the other two the remaining hypophyseal tissue was normal as far as could be determined by microscopic examination. In these two animals, however, there were moderate changes in the thyroids, adrenals and thymus.

Food-intake. All ten animals of the total removal group showed a definite decrease in food-intake, from 20 to 35 per cent. One of the curves is shown in figure 2 *b*. Similar to body-weight, food-intake decreases slowly and remains finally at a permanently low level. Three of the group of partial hypophysectomies showed a decrease, one of these being the animal with the degenerating anterior lobe cells. Moreover, the effect resulting from partial extirpation was temporary, rarely lasting more than a few days. A curve for one of these animals is shown in figure 2 *a*.

Sex cycles. The four-day cycle in activity and in the vaginal smears was absent in the four females in the total removal group. The smears showed the typical dioestrus picture. The five females, in the group in which only the anterior lobe remained, showed normal four-day cycles both in smears and activity. The four-day cycles in running activity can be seen in the curve in figure 1 *a*. The four females, in the group in which remnants of posterior as well as of anterior lobe were found, showed a wide range of variation. One animal showed continuous cornified smears; one, a typical dioestrus smear with no cornified cells; one, a four-day cycle; one, irregular cyclical changes. These data are too few to draw any conclusion relevant to the participation of the various parts of the pituitary in the control of the oestrous cycle.

Water-intake. The effects produced on water-intake by total and partial hypophysectomy are summarized in figure 3 and in the last two columns of table 1. Figure 3 shows five different types of water-intake curves obtained in these experiments. In *a* there is a well-defined large increase to a high level which is fairly constantly maintained. There were four animals with partial hypophysectomy and one with total hypophysectomy in this group. In *b* there is a sharp increase to a very high level, which is maintained only a short time before a new and lower level is established—a level which, however, is still considerably above the pre-operative level. This group consists of three partially hypophysectomized animals. In *c* the water-intake increased for a few days and then returned to its original level. Of this group of four animals, three had had the pituitary gland totally removed, one had had the gland partially removed. In *d* there is no change at all. There were two animals with total extirpation and four with partial extirpation of the pituitary in this group, In *e* there is a decrease in water-intake rather than an increase. There were three animals with partially removed glands and four with totally extirpated glands in this group. These results are summarized in table 1 where it can be seen that only one (no. 595) out of the ten animals with total hypophysectomy showed a

permanent increase in water-intake, although five out of the ten showed a large, but temporary, increase just after the operation. Moreover, inasmuch as five animals with total removal showed, if anything, a decrease rather than an increase in water-intake, it is clear from these results that removal of the pituitary gland does not have a constant or definite effect on the water-intake.

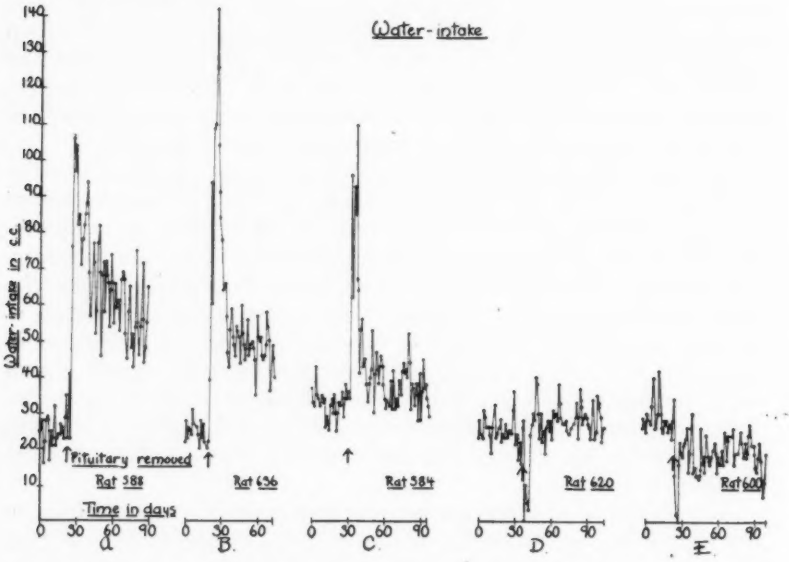

Fig. 3. Graphs showing five types of effects on water-intake produced by hypophysectomy. Water-intake in cubic centimeters is indicated on the ordinates and the duration of the experiment in days on the abscissae. In *a* there is a great increase to a high level which is maintained more or less permanently. In the second type a large increase is present for a short time following the operation, whereupon the water-intake decreases to a lower level which is still, however, considerably above the pre-operative level. In the third type, the water-intake returns to its original level after a sudden, transient increase which persists for only a few days. In the fourth type there is no effect whatsoever upon water-intake and, finally, in the fifth type there is a decrease in water-intake instead of an increase.

It is interesting to note in the table that the partial removal group showed much greater changes in water-intake. Five out of the fifteen showed a permanent increase and twelve out of the fifteen showed a large but temporary rise. We are inclined to attribute the inconstant disturbances in water-intake after complete or partial extirpation of the hypophysis to transitory injuries of the brain-stem incurred in the operation. In some instances undoubtedly a certain amount of trauma to the brain

cannot be avoided, especially when bleeding has been rather extensive. In spite of such an assumption, visible damage to the brain-stem has not been encountered in any of the animals at autopsy.

It may be noted that the magnitude of the increase in water-intake is in some rats quite as large as that which can be produced by a puncture of the brain-stem. The only difference is that on the whole the increase produced by hypophysectomy is only temporary, whereas that produced by actual brain-stem puncture is permanent in all cases.

These results confirm the observations of Dandy and Reichert (1925), Hashimoto (1924), Dott (1923), Bourquin (1927) and others, that complete removal of the pituitary gland does not necessarily produce polyuria. Hashimoto found polyuria in only 5 out of 56 hypophysectomized rabbits. The increase in water-intake which may follow pituitary extirpation is in all likelihood due to an injury of the adjacent tissues of the brain-stem. The amount of injury, however, can be so slight as to produce no apparent anatomical change.

Effects of injection of alkaline extract of the anterior lobe. Eight of the ten animals with the pituitary totally removed and one with the gland partially removed received daily injections of 1 cc. of alkaline extract of the anterior lobe of sheep over a period of 30 to 33 days. These injections produced a very pronounced increase in both body-weight and food-intake, but no effect on either activity or water-intake. The effect produced on body-weight and food-intake can be seen in figure 2 *c*.

It is important to note that the injections apparently did not have any effect on the adrenals, sex-glands and thyroid, for all of these organs were found to be markedly atrophied at the time the animals were killed and only a day or two after the injections were stopped.

In view of the marked atrophy of the adrenals and sex glands, in such hypophysectomized rats it would be interesting to determine whether normal running activity can be reëstablished by the injection of follicular, testicular, or adrenal extracts either alone or in combination.

Description of autopsies. Ten of our rats showed at autopsy that the pituitary body had been completely removed. The brains and meninges of these rats appeared in every respect normal and the passage through which the pituitary had been removed had closed without obvious infection or attendant injury to the brain. No trace of the anterior or of the posterior lobes could be found in these animals. The hypophysis in each instance appears to have separated from the brain, at the time of operation, at the junction of the infundibular stalk with the posterior lobe, for at autopsy the slender infundibular stalk was invariably present and could be traced from the under surface of the hypothalamus to the meagre scar tissue on the surface of the sphenoid bone. The distal end of the infundibular stalk was invariably imbedded in this scar tissue. Thus there appears

to have been in each instance a clean separation of the anterior and posterior lobes of the pituitary with uninvolved healing of the approach and without noticeable injury to the hypothalamus.

On autopsying these rats a series of easily demonstrable changes was seen to have occurred in certain of the other organs, involving principally the thyroids, adrenals, reproductive tract and the lymphoid tissues of the body. Changes in other organs following removal of the hypophysis in the rat have been observed by Philip Smith (1927) and our observations agree in the main with his description. There are, however, certain additional findings to which we wish to call attention.

As in Smith's account, the thyroids and adrenal glands are hypoplastic, the diminution in the adrenals involving solely the cortex. The adrenals are anemic and often extremely minute.

The male reproductive tract is markedly altered. The testes are invariably intra-abdominal and almost completely atrophied, being less than a sixth of their normal size. The accessory genital glands are reduced to minute size. The entire genital tract is anemic, the genital fat has largely disappeared and the remnants of fat that are left have a peculiar brownish color.

The female reproductive tract has undergone similar marked atrophy. The ovaries are small, large follicles are absent and the corpora lutea are variable in size and number. The tubes and uterus are as a rule small, pale and flabby.

One of the striking findings in the present series is a generalized enlargement of the lymph-nodes, enlarged lymphoid follicles of the intestinal tract and the presence of a large thymus gland. The spleen does not appear to share in this general lymphoid hyperplasia. The lymphoid hyperplasia is stressed because of its possible relation to the atrophied adrenal cortex. Crowe and Wislocki (1914) have observed a similar generalized hyperplasia of the lymphoid tissue of the body following reduction of the adrenal cortex in dogs by surgical removal. Similarly Jaffe (1924a, b) has described thymus hyperplasia or regeneration following suprarenalectomy in the rat. There appears to be some basis, therefore, for assuming in the present experiments that the tremendous enlargement of the thymus and lymph-glands is associated with the pronounced changes in the adrenal cortex.

Aside from the above changes the only other general feature of the picture produced by hypophysectomy in our series is a rather widespread disappearance of body fat, especially noticeable in the fat deposits around the viscera. The striated musculature of the body, on the other hand, does not appear to have wasted. As Smith has shown in the rat, obesity is characteristic of injury to the tuber cinereum and hypothalamus, but does not follow removal of the hypophysis.

Of the fifteen rats in which hypophysectomy was incomplete, three showed moderate changes in the other organs—large thymuses, small adrenals and thyroids. These three (nos. 597, 584, and 634) showed clinically a marked reduction in activity. In one of these (no. 597) upon microscopic examination the remaining anterior lobe tissue was found to be degenerating.

Microscopic examination of the remaining hypophyseal tissue in the other fourteen rats showed healthy, well-vascularized anterior lobe tissue. In each of these rats the remaining pituitary tissue amounted to from one-quarter to three-quarters of the original gland. In five instances a portion of the posterior lobe and pars intermedia remained as well as anterior lobe. Microscopic examination revealed that the remaining anterior lobe tissue consisted of easily identifiable basophilic, eosinophilic and chromophobe cells, with the exception of two (nos. 600 and 620) in which no eosinophilic cells were encountered. In rat 620 a small epithelial mass composed solidly of cells, resembling a small adenoma, had grown in one area.

SUMMARY

1. The hypophysis was completely removed in ten adult rats and partially removed in fifteen adult animals, leaving intact either parts of the anterior lobe, or parts of both anterior and posterior lobes.

2. The ten animals with total removal of the glands were very inactive, as was determined by means of records of running activity in revolving drums. Spontaneous activity was not affected by partial removal of the gland.

3. Body-weight and food-intake were definitely decreased by hypophysectomy. Usually they decreased to a low level, at which they remained fairly constant.

4. The four-day sex cycles were absent in the hypophysectomized animals, present and normal in the animals with only anterior lobe tissue remaining. In three animals, with both anterior and posterior lobe tissue present, the sex cycles were markedly disturbed.

5. Permanent polydipsia was produced in a large fraction of the rats with the pituitary gland partially removed, but occurred in only one out of ten animals with the gland totally extirpated. Further evidence has been gained that polydipsia does not depend on the removal of the pituitary gland.

6. The variable and often transient increase in water-intake following complete or partial hypophysectomy is presumably due to the injury of the adjacent brain-stem. The magnitude of the increase is often the same as that characteristic of the increase produced by puncture of the neighboring tuber cinereum, but the increase is transient.

7. Injections of alkaline extract of the pituitary gland following hypo-

physectomy produce a definite increase in body-weight and food-intake, but have no effect on spontaneous activity or water-intake.

8. In completely hypophysectomized rats, autopsy reveals marked changes in other organs and tissues. The thyroids and adrenals are hypoplastic. The reproductive organs are atrophic. The thymus and the lymph-glands of the entire body are markedly enlarged. There is a pronounced disappearance of body fat. These changes can be obtained in a mild degree, in some instances, after incomplete removal of the pituitary.

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THE RENAL TUBULE AND GLOMERULUS

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In preceding studies of the writer (1926, 1927, 1928, 1929) emphasis was confined chiefly to the secretory activity of the tubule. An associated observation, however, on the concentration of dyes in the distal convolution of the frog's kidney was made in 1924 and subsequently referred to (Edwards, 1926, p. 333) as follows: "The appearance of the dye in concentrated form in the lumina of the distal convoluted tubules after the experiment has proceeded for a time, is in some way conditioned by the method of experiment." This observation has meanwhile been held in reserve pending further work.

Bieter and Hirschfelder (1924) prior to the writer had figured the concentration of phenol red in the lumina of the tubules of the frog's kidney. They say concerning this concentration: "The color of the dye is very deep in the convoluted tubules, and seems to deepen progressively as one passes from the proximal to the distal loops. This would indicate that concentration begins in the proximal convoluted tubules." But in 1929 (p. 198) they make this statement: "Bieter and Hirschfelder (1924) were the first to point out that dye solutions, being excreted by the living frog's kidney, were concentrated in the tubules on the ventral side of the kidney, which are the distal convolutions." This is misleading since nowhere in the 1924 publication is mention made of dorsal or ventral surfaces nor is there the slightest indication that these authors knew that there is a difference between the segments of tubules visible on these surfaces.

White (1926) held that in the amphibian, Necturus, sugar and chloride were reabsorbed in the proximal convolution and in 1929, "that there is no significant exchange of water between the tubule wall and lumen." However, no valid proof of the above has as yet been forthcoming.

Richards and Barnwell (1927, p. 79) state that "the level of the tubule at which the concentrated dye collects cannot as yet be stated in terms of its known morphology."

Bensley and Steen (1928), present convincing data concerning the concentration of certain dyes in the distal convolution of the frog's kidney and also give Nussbaum (1886) credit for this original observation. They state (l. c., p. 89) that "in the tubules of the normal kidney of the frog, the

events after injection of indigo carmine into the submaxillary lymph sac, are similar to those in the kidney with ligated arteries, modified first by the addition of the glomerular product and secondly, by the greater activity of the glandular epithelium. The rate of secretion is faster and the solution is swept along the tubule at a more rapid rate, and is less concentrated in the distal tubules than in kidneys or areas where the renal arteries are tied." This statement is true despite the fact that the observations constituting its basis were made on pithed but otherwise intact frogs as compared with similarly treated ones with ligated arteries. An equally convincing demonstration can be had by injecting any readily excretable, easily observable, non-toxic dye simultaneously into two frogs and after an hour or more preparing one frog vivisectionally for microscopic study of its kidneys and the other after pithing with destruction of the cord.

The present study was undertaken with the three-fold purpose of ascertaining: 1, the conditions under which certain dyes, during their passage through the kidney, are or are not visibly concentrated within the renal tubule; 2, the precise locus within the tubule where this concentration occurs; 3, the significance (aside from the possible content of urinary constituents) of *a*, the constant release of fluid from the glomerulus when active and *b*, the mechanical and other possible effects of the pressure thus exerted on the epithelium of the tubule.

For this purpose animals representative of three classes were used: 1. Amphibia. Male frogs (Rana pipiens) weighing 20 to 30 grams. 2. Reptilia. Female turtles, weighing 400 to 500 grams. 3. Mammalia. White, male rats weighing 80 to 100 grams. Beginning in 1923 the kidneys of all of these animals as well as others have been previously studied by the writer in an effort to obtain comparative data by means of which might be made clearer the functional significance of certain segments of the renal tubule whether in meso- or metanephroi, in cold- or warm-blooded animals, in fish, salamander, frog, turtle and rat.

RESULTS. I A. *The conditions under which substances are visibly concentrated in their passage through the tubules of the living frog's kidney.* The general condition for such concentration is that leading to partial or total failure in the circulation of blood through the glomeruli. The experimental conditions under which dyes (phenol red, tetrachlorphenolsulphonephthalein, buffalo fast crimson) are visibly concentrated in the lumen of the distal convolution when observed under the microscope, following their preoperative injection (0.5 cc. of a 0.6 per cent solution) into the dorsal lymph sac are: *a.* Destruction of the brain and cord. *b.* Conspicuous hemorrhage or weakened heart beat (experimentally induced by puncture of the ventricle or by pinching it adequately with forceps). *c.* Motor paralysis following a dorsal lymph sac injection of 3 to 4 minims of a 2 per cent solution of curare. *d.* Ligation of one renal artery (the frog

has 5 to 6 per kidney) of either a normal or diuretic kidney. Visible concentration of the dye appears within 3 to 5 minutes or less after ligation and continues until the ligature is removed and circulation restored. This observation may prove elusive for those not adept in the use of the microscope since the lumina of the tubules are rapidly reduced—frequently to a mere slit in which the content of dye may be difficult to observe unless at a magnification of several hundred diameters (fig. 15, left, triangular portion of picture, 16, 18). *e.* Coagulation by heat of one or more active glomeruli in the normal or diuretic kidney. The dye is concentrated in the lumen of the distal convolution of the tubule with which the inactivated glomerulus is connected. Coagulation is accomplished by applying for a second or less to the periphery of a given glomerulus the tip of an extremely fine-pointed platinum wire whose other end is welded to a thermocautery. The glomerular area covered was approximately one-half its diameter. A glomerulus thus inactivated is easily and permanently distinguishable by its ashy gray color to which is added the faint and uniform color of the dye used.

I B. *The conditions under which the dyes just mentioned are not visibly concentrated in their passage through the tubule of the living frog's kidney.* *a.* In the kidney of the healthy, unanesthetized, unpithed frog when it is observed under the microscope following its exposure by vivisectional operation whether the dye is injected previous to the dissection by way of the dorsal lymph sac or intravenously (anterior abdominal vein) after the dissection. *b.* Similarly injected dyes are not concentrated by the diuretic kidney whether the diuresis be caused by urethane, intravenously injected Ringer's solution or neutral salines. Other diuretics were not used. *c.* The observed kidneys of frogs injected with dye show under a variety of experimental conditions variously active glomeruli. Tubules attached to the poorly active or inactive glomeruli of such a kidney stand in marked contrast to tubules connected with fully active glomeruli owing to the presence of concentrated dye in the lumina of the distal convolution of the former.

Since the results thus far presented were obtained from experiments on frogs, their validity is accordingly restricted to this animal. In order to see to what extent comparable results might be obtained in other vertebrates, turtles and rats were selected. The method of experiment was designed to reveal with certainty, first, what segments of the renal tubules of these animals are visible on the surface of the living kidney and second, the degree of visible concentration of dyes and iron salts in them. The first is excellently accomplished by macerating the kidney of both turtle and rat in 37 per cent HCl for 18 and 20 hours respectively and then washing in several changes of distilled water. The surface of the kidney best suited for microscopic study in the living animals is also chosen in the

macerated kidney and the tubules teased apart in this area. The relation of each segment or convolution to the surface studied is thus established. The second by intraperitoneal injections (10 cc. for turtle and 2 cc. for rat) of equal parts of a 1 per cent solution of ferric ammonium citrate and sodium ferrocyanide. Intraperitoneal injections were made in the turtle by boring a very small hole through the center of the plastron and then inserting a hypodermic needle connected with a syringe through this opening into the body cavity. If necessary after the injection the opening was sealed over with bone wax. After several hours (5 or more for the turtle and 3 for the rat) the animals are treated as follows: *1. Turtle.* The animal is held in one hand and a trephine placed on the dorsum of the carapace and directly over either right or left kidney. A hole is bored through the shell and the thin layer of softer tissue removed by scissors and cautery. The kidney is rapidly excised and placed in 37 per cent HCl. The other kidney is removed (in 15 minutes) and put in 5 per cent trichloracetic acid for 24 hours after which it is kept permanently in 95 per cent alcohol. By means of this latter preparation comparisons were made from time to time of the segments of the tubules in which dyes (chiefly tetrachlorphenolsulphonephthalein) are concentrated when injected intraperitoneally and the segments of the tubules in the living kidney subsequently observed under the microscope. Following some of the iron salt injections, the turtle's head was crushed, the plastron removed and the kidneys fixed *in situ* either in HCl or trichloracetic. For microscopic study of the living kidney (3 to 5 hours after the injection of the dye) it was always exposed by trephining through the shell of the dorsum as described. This method is not adapted for continuous study of the kidney, but is entirely adequate for determining the segment of the tubule in which dyes are concentrated. *2. Rat.* It is given a light ether anesthesia, an incision is made in the abdomen and the kidneys exposed and fixed *in situ* either in HCl or trichloracetic. For microscopic study of the living kidney, urethane anesthesia was found preferable.

II. *The locus within the tubule where the concentration of dyes and iron salts occurs. 1. Dyes* (4 experiments with tetrachlorphenolsulphonepthalein and 2 each with phenol red and buffalo fast crimson,—10 cc. of a 0.6 per cent solution) when injected intraperitoneally into the turtle and the living kidney is subsequently observed under the microscope, are seen to be uniformly distributed in the cells and lumen of the proximal convolution and progressively concentrated only in the lumen of the distal convolution in a way entirely comparable to that depicted in figure 2. The hemorrhage following the operation due to the intermittent pressure of the lungs against the kidney and it against the carapace and other factors suffice apparently to induce this visible concentration. More elaborate experiments such as described for the frog have not as yet been undertaken. In the rat

after intraperitoneal injections of the dyes mentioned (2 cc. of 0.6 per cent solution) and exposure of the kidney following urethane anesthesia, they were not appreciably concentrated until the experiment had continued for 1 or more hours. In areas of local stasis due sometimes to decapsulation, without which microscopic observation of the tubule is obscure, and sometimes to gradual circulatory failure in the kidney owing to its exposure and length of the experiment, the dye in the lumina of the distal convolution became more markedly concentrated. Hence the inference seems safe that in the turtle and rat as in the frog, marked, visible concentration of dyes does not occur under normal conditions. Maximum concentration of dyes and iron salts in frog, turtle, and rat has occurred by the time these reach the lumen of the *distal* part of the distal convolution. In the collecting duct of frog and turtle and in the junctional tubule and collecting duct proper of rat or in any ramification of the collecting duct system, no further intensification of dye is observable. *2. Iron salts.* If no further experiments had been performed than those responsible for the results reported above, especially those reported for the frog, the conclusion would seem sound that the concentration of dyes ocurs only under abnormal conditions and therefore that the differences in the concentration of urinary constituents as determined in plasma and bladder urine, might be the result (in some cases at least) of the passage of a given constituent through the tubule epithelium in a certain concentration such that it reached the bladder thus concentrated, or modified by mixing with glomerular fluid. Fortunately, more reliable indications are available from the study of the distribution of iron salts within the tubule.

In normal or in diuretic frogs, turtles and rats, the injection of iron salts is followed by a definite though variable concentration of them within the lumen of the distal convolution (figs. 1, 2, 3). The locus of this concentration as shown in figures 1 to 3 was determined by first macerating the respective kidneys of these animals following intraperitoneal of the iron salts. Then the tubules of such kidneys were teased apart and studied under the microscope. Representative tubules of the thousands so isolated for each type of kidney are shown in figures 4 to 14.

It would appear that the mutual check supplied by dye and iron salt excretion is a valuable one and not least as regards the condition under which there is observable and variable dye excretion when these are carefully recorded and reduplicated following the injection of iron salts. Any given stage can be preserved by putting one kidney of an animal used in a given experiment in trichloracetic and the other in HCl. In the macerated tubule when teased out and fixed permanently to a substrate (cf. figs. 4–14) are revealed the differences in segmental activity occurring up to the time of fixation. The kidney in trichloracetic preserves the differences as seen on the surface.

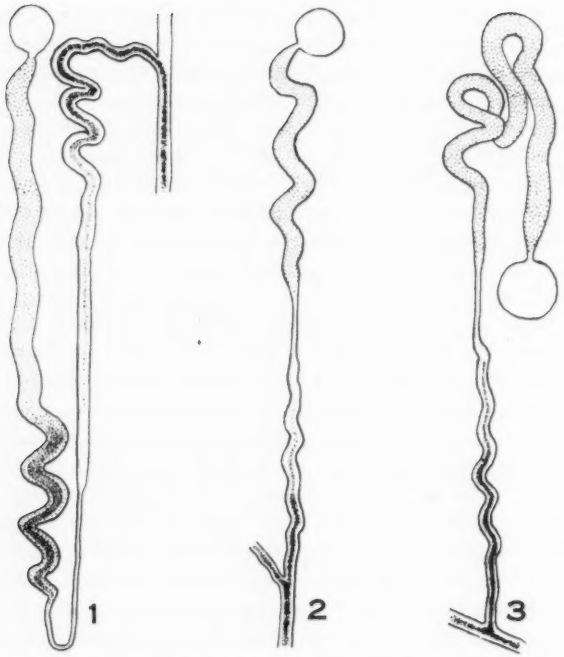

Fig. 1. Diagram of a renal tubule of the white rat showing circular capsule, proxima convolution, spiral descending limb, thin segment, ascendng limb, distal convolution, junctional tubule and duct. The light stippling of the proximal convolution indicates the presence in cells and lumen of dyes and iron salts (concerning the latter, see text). The former as seen in the cells of the proximal convolution in the living kidney, the latter in this convolution of the isolated tubule following maceration in HCl of a kidney actively excreting the salts at the time it was immersed in the acid. The heavier stipple in the lumen of the descending limb is employed to indicate the presence of larger amounts of Prussian blue than occur elsewhere in the lumen of the proximal convolution. The color and size of these particles do not indicate the result of appreciable dehydration such as is seen in the lumen of the distal convolution. The progressively heavier stipple in the lumen of the ascending limb and distal convolution represents larger and much bluer masses, presumably the result of marked dehydration beginning in the thicker portion of the ascending limb.

Fig. 2. Similar diagram of an isolated tubule from the macerated kidney of a turtle previously injected with iron salts showing by stipple, as noted above for dyes and apparently iron salts, their uniform distribution in cells and lumen of the proximal convolution, their absence from the narrow, intermediate segment and their progressive, luminal concentration only in the distal convolution.

Fig. 3. Similar diagram showing the distribution of iron salts in the tubule of the frog's kidney. Note in this tubule and in the two just described the marked similarity in convolutional distribution and concentration of Prussian blue. The same is true of readily excretable dyes when observed in the living kidney.

IIIa. *The significance of the constant escape of fluid from the glomerulus when active. 1. The flushing action of the glomerulus.* Dyes injected into a frog previous to microscopic observation of its kidney are subsequently observed to become concentrated in the lumen of the distal convolution. The degree of observed concentration appears to vary directly with the variation in glomerular blood flow and the consequent glomerular fluid issuing from the capsule. The concentration is less with increased flow and vice versa. In frogs' kidneys the tubules of which are markedly concentrating dye, i.e., ones with slow or inactive glomeruli, even to the extent of precipitating it (see Bensley and Steen, l.c.), the dye is washed out to the point of invisibility in 3 to 5 minutes after the intravenous or intraventricular injection of Ringer's solution. In several tubules, however, and in a large number of experiments, it was noted that the dye had attained an extreme concentration and was static within the lumen of the distal convolution. When Ringer's solution was injected, the inactive glomeruli connected with these tubules became active and almost simultaneously the narrowed lumen of the tubule began to dilate. That this dilatation is the result of the flow of fluid through the tubule is shown by observing the concentrated column of dye become separated from the walls of the tubule on all sides, and as the relatively clear fluid rushes past between this static column of dye and the tubule wall, it becomes tinged, cuff-like, around the dye, while the fluid next the wall remains untinged. Finally, of course, the dye is washed entirely from the lumen. Observed under the microscope immediately after the injection of Ringer's solution, an increase in blood flow occurs with resumption of flow in the inactive glomeruli. Since by the method of iron salt injection and maceration, the precise relation of a tubule to its glomerulus for all the tubules along the ventral margin of the kidney is known, no doubt exists regarding the sequence of events when an inactive glomerulus becomes active. In no observed case does the dye begin to wash out in the tubule before the glomerulus connected with this tubule becomes definitely active. The movement of the dye is then extremely rapid, so rapid in fact that continuous observation is necessary to record the disappearance of the concentrated dye in the tubules previously observed and counted. After a brief period, depending on the amount of Ringer's solution injected, many glomeruli become inactive again and the same picture of luminally concentrated dye again appears. Another injection of Ringer's solution leads to a recurrence of the rapid washing out of the dye.

2. *Making an individual tubule aglomerular in the normal kidney.* As a result of the studies of Bensley and Steen (l.c.) and the more recent ones of MacKay and Oliver (1930) and Oliver and Shevky (1930a) considerable interest attaches to the following experiments. Frogs were anesthetized with urethane (1 cc. of a 10 per cent solution via the dorsal lymph

sac). In one series of experiments, 0.5 cc. of a 0.6 per cent solution of tetrachlorphenolsulphonephthalein was injected along with the urethane. After anesthesia and exposure of the kidney certain very active glomeruli were selected from among those visible on the ventral surface. The finely pointed, platinum tip attached to a thermocautery was momentarily applied to the periphery of one or more of such glomeruli. Within 15 to 30 minutes a marked concentration of the dye appeared in the lumen of the distal convolution. If 10 or more glomeruli are cauterized at about the same time in the same kidney, the appearance of the dye in concentrated form varies in the lumina of the various distal convolutions of the tubules connected with these inactivated glomeruli. This seems clearly to be the result of 1, the variable width of the lumina of these tubules; these are so variably reduced in width after glomerular inactivation, that some of them appear as irregular fissures so small that the dye present may be easily overlooked; 2, the rate of secretion by the cells of a given proximal convolution evidently varies (Bensley and Steen, l.c., p. 91) and is independent to a certain extent of glomerular activity. That the aglomerular tubule functions at different rates is indicated by Verne (1922) in his splendid paper dealing with the development, structure and function of the aglomerular kidney.

Another series of experiments was performed on frogs anesthetized with urethane, the kidneys of which were thereby made diuretic, but the dye was not injected until after the kidneys had been exposed and a selected number of glomeruli cauterized in each. Marked concentration of the

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Plate I. Photomicrographs of isolated tubules from the kidneys of rat, turtle and frog (× 32, except 12 and 13 which are × 80).

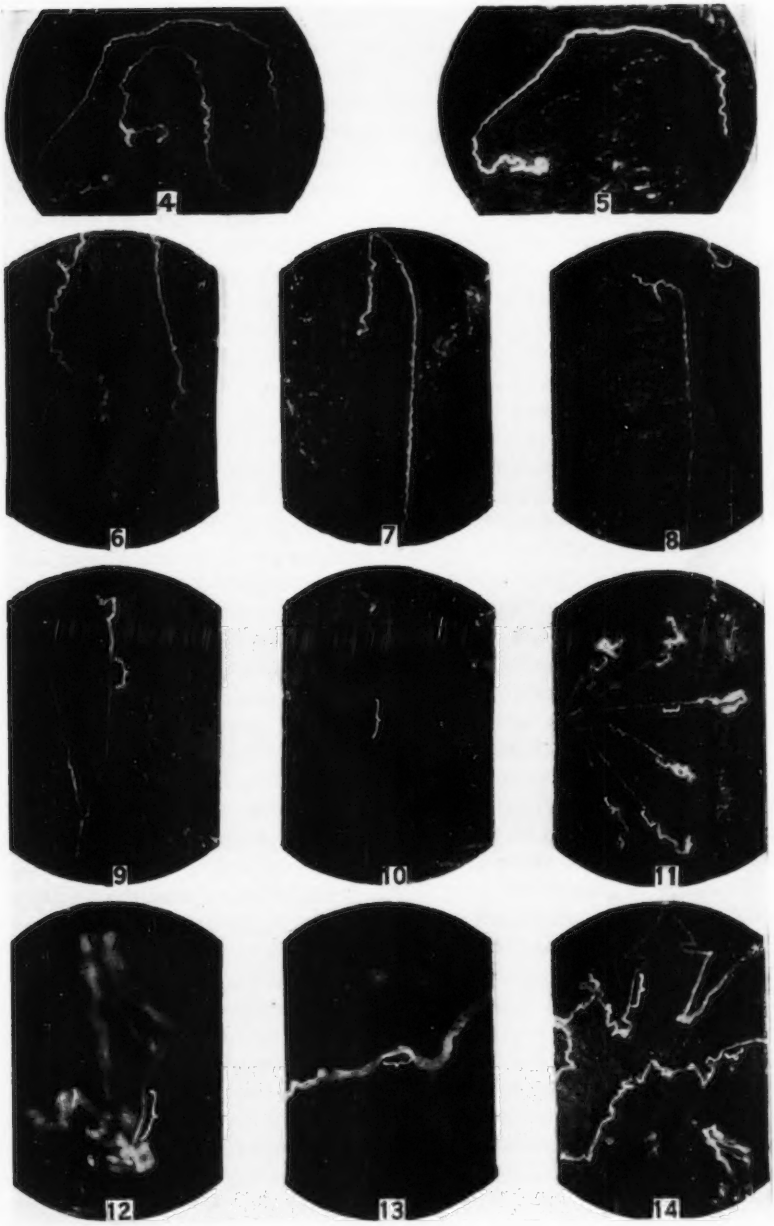
4, 5, 7. Proximal convolutions of tubules from the rat's kidney.

6. A part of the proximal convolutions and the descending limbs of two tubules from the rat's kidney. Note at the lower end of the tubules at the left the beginning of the thin segment.

8. Ascending limb, distal convolution junctional tubule and collecting duct of the tubule of the rat's kidney.

9, 10, 11. Tubules from turtle's kidney. Above the brackets in the first 2 figures and to their right in figure 11, are seen proximal convolutions. Portion of tubule bracketed is the intermediate segment. Below brackets (and to left, fig. 11) are seen distal convolutions joined to collecting ducts.

12, 13, 14. Tubules from frog's kidney. In figure 12 is seen the tubule as coiled normally within the kidney. In the upper half of this picture is seen the proximal convolution; in the lower center opposite bracket, the glomerulus; and in the lower half, the distal convolution. The extent of the intermediate segment is bracketed. Note in this bracketed segment and in the one shown in figure 13, its lighter appearance. This is owing to the presence of long cilia within the lumen. Note in figure 14, upper half, the glomerulus at the right and the proximal convolution tapering from top center to bracket where the intermediate segment begins. The distal convolution is at the left of the bracket. In the lower right of figure 14, is seen the tubule which was photographed at greater magnification in figure 12.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dye in the lumina of the distal convolution of these tubules with inactivated glomeruli occurred at intervals varying from 20 to 40 minutes. These are readily observed under a binocular microscope at a magnification of 30 diameters. More refined observations at higher magnification indicate a shorter time for the initial appearance of the dye in concentrated form. The proximal convolution was also observed and seen to be colored as regards cells and lumen earlier than the dye appeared in the distal convolution. The cells of the distal convolution have in no instance been observed to be colored either by dyes or iron salts when the presence of the latter was studied in the macerated tubule or in section of the control kidney fixed in trichloracetic acid. Experiments are to be perfomed in which tubules with inactivted glomeruli are studied following glomerular inactivation and subsequent injection of iron salts. Such experiments should prove conclusive as to whether the tubule can eliminate these salts. Experiments wherein a single tubule is made functionally aglomerular in a highly active kidney, surrounded as it is by normal tubules with adequate blood supply, and found to be capable of secreting in the proximal convolution and concentrating in the distal, would appear to have more validity than those variously reported since Heidenhain's.

The observations of Oliver and co-workers are of peculiar interest here. Oliver, following Höber (1927), accepts Höber's notion of tubular damage by various agents among which is found urethane. Oliver used for his experiments on Rana catesbiana a perfusion fluid containing 2 per cent urethane. This concentration is about the same as or less than would obtain in the blood of a 20-gram Rana pipiens anesthetized with 1 cc. of a 10 per cent solution of this hypnotic. In no instance, however, has the writer observed the slightest reduction in the intrinsic concentrating power of the cells of the distal convolution. Even when the frog has been kept under urethane anesthesia for two days and continuously excreting previously injected dye, any hindrance, general or local, to the blood flow through the glomeruli is quickly followed by concentration of the dye in the lumen of the distal convolution. Again, when iron salts are injected into a frog under urethane anesthesia and a maximum diuresis maintained by immersing the frog in Ringer's solution made isotonic with the injected urethane, a definitely greater amount of iron is found precipitated in the lumina of the distal convolutions than elsewhere in the tubule proper (when the kidney is macerated in HCl) but markedly less than in a non-diuretic kidney or in one so treated in a pithed frog. The situation in this type of diuretic kidney seems clearly to be that the release of fluid, chiefly from the glomerulus, prevents the occurrence of the normally more marked concentration in the distal convolution of the non-diuretic kidney. There is no evidence from these studies that urethane injures the tubule in any significant way. The sugar that Oliver finds in his perfusion urine is

probably not the result of damage, but eventually the cause, owing to a not adequate absorption of this possibly important constituent for renal cell metabolism.

Oliver also disagrees with the writer concerning the excretion of neutral red. The writer found that Rana pipiens does not excrete in appreciable amounts this dye when it is injected via the dorsal lymph sac. Doubtless the same would be true if Rana catesbiana were injected with this dye in the same way.

From 1 and 2 above it is seen that when the glomerulus is active, the lumen of the tubule is bathed by a fluid which keeps it free of too concentrated substances. In the proximal convolution there is no indication that the concentration of such substances is the result of reabsorption. In the distal convolution the evidence seems inescapable that it is the result of a very marked and rapid reabsorption of water. To what extent the washing function of the fluid escaping from the glomerulus is coupled also with the normal excretion of urinary constituents and perhaps more importantly with the intrinsic metabolism of the cells of the tubule, is not clear. That a tubule once connected with its glomerulus cannot long maintain its functional or structural integrity without it, is a common observation. That the lumina are narrowed and a coagulable or precipitable exudate appears in them is generally seen in tubules with inactive or inactivated glomeruli (cf. the narrow and wide lumina of tubules shown in fig. 15, and fig. 17 with 16 and 18).

IIIb. The mechanical effect of the pressure exerted on the renal epithelium owing to the passage of fluid through the lumen of the tubule. Many investigators have been interested in changes in renal epithelium following or associated with changes in renal activity. Cushny (1926, p. 7) dismisses the whole matter by saying: "Hitherto no changes have been shown to occur during activity in the kidney except those arising directly from the distension of the capsule and tubules with fluid." While this is probably true, it was of interest to the writer to determine the effect of pressure on the epithelium of the tubule when various media are injected by way of the ureter. Adequate indication is afforded by figures 19 to 22. The kidneys of frogs used in these experiments were allowed to become almost non-functional in order to obtain maximum effects. Certain specificities are revealed by the use of the various media injected. The more interesting observation afforded by these experiments is the rapidity with which Ringer's solution and to a less extent, glucose, when injected via the ureter, are apparently taken into the blood stream. This is best seen following the injection of Ringer's solution. Within a minute the heart action and circulation are improved to such an extent that inactive glomeruli resume function. By continuous, very slow injection a condition of stasis can be removed and the circulation in the kidney made to appear as

normally observed. With discontinuance of the injection, the circulation soon returns to its former state. That the preceding may be the result of a pyelovenous back-flow is indicated by the following, namely, it was noted during the injection of several media and especially of mineral oil, that the terminal capillaries, venules and larger branches of the vena cava became progressively and rapidly injected with oil. No record was made of the pressure applied during the injection since the latter was stopped and the kidney fixed *in situ* as soon as the visible glomerular capsules were adequately distended, thus indicating for the purpose of the experiment, that the tubules also were injected throughout their length. Further experiments are in progress to determine the nature and route of what seems clearly to have been the result of a pyelovenous back-flow.

Discussion. Since the writer's initial (1923) but hitherto unpublished studies on the excretion of dyes by the living kidney of the unanesthetized turtle, and his subsequent and similar studies on the rat, frog, Necturus and the aglomerular fish kidney, indisputable evidence has accumulated showing the active elimination of certain dyes by the tubule. The segment of the tubule in which this elimination takes place is now demonstrably the proximal convolution as observed in fish, amphibia, reptilia and mammalia. In this final determination of the secretory function of the tubule (not convincingly if at all defined as proximal convolution) many investigators variously share. It is interesting to note here how reabsorption in the past was believed to occur in the proximal convolution primarily because the striated, luminal border of this convolution resembles strikingly that of intestinal epithelium. Proof of the reabsorptive power of this convolution does not appear conclusive.

The determination of a secretory function of the proximal convolution invites attention to certain results obtained by Richards and White. The

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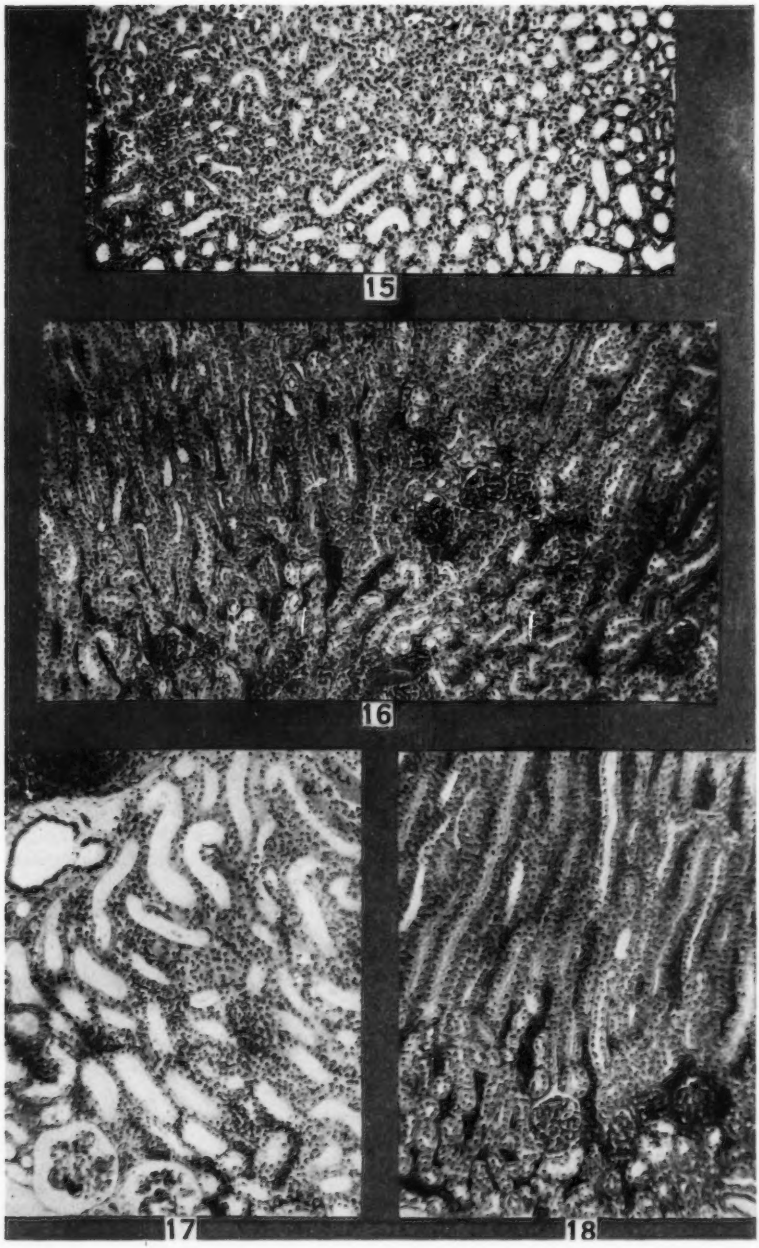
Plate II. Photomicrographs of sections of frogs' kidneys (× 80).

15. Section of a kidney of a frog anesthetized with urethane. The kidney was fixed in situ 2 hours after ligation of one renal artery. In the upper left of the picture is shown the area normally supplied by the ligated artery. Note in this area the taller epithelium and contracted lumina of sections of tubules containing varying amounts of coagulated substance. The opposite is seen in the lower right of the picture. The blood supply to this area was left intact.

16. Section of a kidney of a frog given 4 minims of a 2 per cent solution of curare 9 hours previous to in situ fixation. At the time of fixation, all visible glomeruli (50) were inactive and the heart beat had been reduced from an initial rate under curare of 38 to 24.

17. Section of a frog's kidney made diuretic by a dorsal lymph sac injection of urethane 3 hours prior to fixation. Note dilated lumina, low epithelium of the tubules and the 2 distended glomerular capsules.

18. Section of a frog's kidney initially diuretic as in picture 17. Ventricular puncture made 3 hours later caused hemorrhage, a slightly reduced rate and a markedly reduced force of beat. In situ fixation 2 hours after puncture.



former found in the glomerular fluid of frogs by microchemical analysis a close approximation to a plasma filtrate; but chiefly or only when the lumen of the tubule is occluded from its glomerulus near the neck segment, and oppositely when the lumen is patent. White, however, interprets the laking of the red cells of the dog when injected via the capsule into the lumen of the proximal convolution of the tubule of Necturus, as the result of a hypotonicity brought about by the reabsorption of chloride from the lumen of this convolution.

In order to harmonize the findings of these two investigators, a differential reabsorption of water and chloride from this convolution would have to occur whereby glomerular fluid could at times become a hundred per cent more concentrated (owing ostensibly to luminal withdrawal of dehydrated chloride) and at the same time be hypotonic enough to lake mammalian red cells. This situation seems paradoxical and none the less so since Bieter and Hirschfelder, the writer, Richards and Barnwell, and Bensley and Steen have all presented data showing the consequence of what appears clearly to have been the result of water reabsorption. The locus of this reabsorption is chiefly or solely the distal convolution as shown by the writer for a variety of vertebrate kidneys, by Bensley and Steen for the frog and by Bensley for the rabbit (personal communication of results in press).

The determination of the proximal convolution as the sole observed site for the secretion of dyes in the vertebrate kidney including its precise equivalent in the aglomerular kidney (Edwards, l.c.) and similarly the distal convolution for the reabsorption of water owing to the visible concentra-

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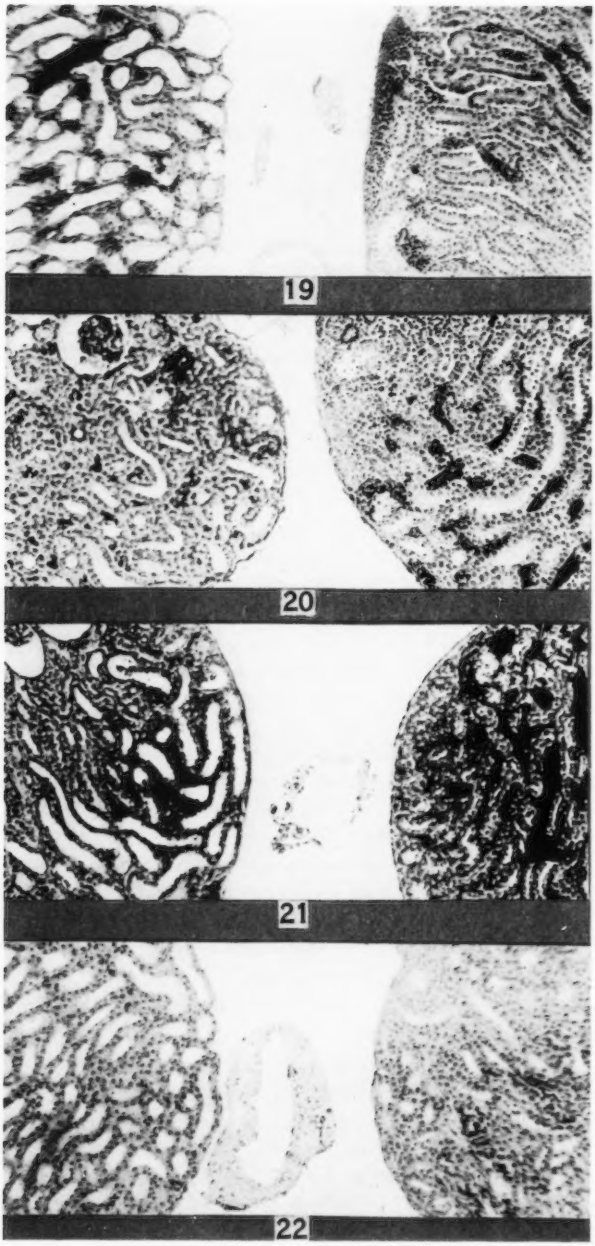
Plate III. Photomicrographs of sections of frogs' kidneys showing the effect on the tubule epithelium of ureteral injections of various substances. Controls are shown at the left of the pictures (× 80).

19. Section of right and left frog's kidneys fixed 24 hours after frog was anesthetized with urethane and immediately after ureteral injection with mineral oil of kidney shown at the right of the picture. Dark areas between tubules represent blood in stasis when fixation occurred.

20. Similar section showing, at the right, the effect of an ureteral injection of 5 per gum acacia solution. Frog given by injection into the dorsal lymph sac, 3 minims of a 2 per cent curare solution 8 hours before cannulation of ureter. Kidney fixed 4 hours later. No circulation was observed in the kidney during the time of ureteral cannulation, injection and fixation.

21. Section of frog's kidneys showing the effect of an ureteral injection of a 10 per cent glucose solution. Frog kept under urethane for 13 hours before ureteral injection and fixation. Note marked stasis and collapse of tubule lumina in the picture at the right. This stasis was caused by a weakened and slow heart beat.

22. Section of frog's kidneys showing the effect of an ureteral injection of Ringer's solution, 2 hours after the frog had been given 0.25 cc. of a 40 per cent urethane solution by dorsal lymph sac injection. This concentration of urethane caused marked ascites, laking of the blood cells and inactive heart. The frog was still capable of leg reflexes and a vigorous peristalsis was observed in the intestine and bladder.



tion in the lumen of this convolution of dyes and iron salts, adds appreciably to the limited number of established functions of the tubule. If there be reabsorption of water or solids in the *proximal* convolution of the renal tubule of any vertebrate kidney, the proof, it appears, is hardly conclusive, although the writer is not prepared to contend that such does not occur.

Another point of interest concerns the process involved in the passage of water or solids through the epithelium of the tubule in either direction. A certain consensus of opinion indicates two possible mechanisms: one passive, the other active, occurring simultaneously or alternately in either proximal or distal convolution or in some unpostulated part of the tubule. It appears doubtful that any significant exchange of water or solid is accomplished in the kidney by a passive process.

The remarkable effectiveness of glomerular fluid in washing out the lumen of the tubule has been noted. The presence of exudate in the lumina of tubules with inactive glomeruli as well as those of aglomerular kidneys is consistently observed. The lumina of the glomerular tubules of marine fish kidneys contain considerable exudate apparently the result of some relative inadequacy in glomerular function. This exudate is more conspicuously seen under appropriate conditions in the lumen of the proximal convolution or its equivalent, of the tubules of all the vertebrate kidneys studied. Perhaps Bowman was more nearly correct in his theory of renal function than other investigators have been since.

The visible mechanism for the propulsion of urinary constituents through the aglomerular fish tubule is strikingly absent when such a tubule is compared with glomerular tubules of fish living in essentially identical environments. The former is without ciliated or constricted segments and the brush border of the cytological equivalent of the proximal convolution, is generally lower than in any glomerular, vertebrate tubule studied. The presence of a distal convolution comparable with certainty to that of higher vertebrates has not been found (Edwards, 1929). Just why the aglomerular tubule should be relatively so simple in structure and the glomerular ones of fish, living as indicated, should in some instances possess ciliated, brush bordered or constricted segments is not at present clear. The passage of glomerular fluid (aside from the stimulus responsible for the development of the glomerulus) through the tubule evidently affords a new stimulus for the tubule both as regards its cytological and morphological structure (Edwards, 1929).

That the volume of urine excreted per day by aglomerular as compared with glomerular fish kidneys varies in a way suggestive of differences in blood supply to these kidneys, has already been noted by the writer (Edwards, 1928). Marshall and co-workers (1930, report by the former at the renal symposium, Minneapolis, July 7–18) as a result of supplementary studies on marine and fresh water fish as well as higher vertebrates, suggest

that the ciliation of certain segments of the renal tubule of cold-blooded vertebrates is in response to the relatively low blood pressure obtaining in such vertebrates and also the consequent need for a more effective water elimination. The latter need is indicated by a comparison of the kind of glomerular development found in marine and in fresh water fish and in the higher vertebrates. With such glomerular development is associated by Marshall and co-workers the difference as regards the hypo- and hypertonic urines of cold- and warm-blooded animals respectively. This difference was significantly noted by the writer in an earlier publication (Edwards, 1928).

However, the problem with which the writer originally set out, namely, the determination of the place of transfer of urinary constituents from blood to tubule lumen, and which has led him, essentially unaided, to attack this problem by comparative studies, is now in certain respects reduced. The idea for this mode of attack was explicit in renal literature long before the writer entered the renal field, yet it remained for him to inaugurate with practical results, comparative renal studies.

The vertebrate tubule can eliminate in part or entirely and variously concentrated, all or most of the normally occurring mammalian urinary constituents. The process and site are likely the same in all tubules. The glomerulus appears not so much the chief locus for the elimination of urinary constituents as that it affords a means for the maintenance of the integrity of the tubule.

SUMMARY

1. Data are presented showing the locus of concentration of dyes and iron salts within the lumen of the distal convolution of the renal tubule of three classes of vertebrate kidneys.

2. The site of water reabsorption is indicated by the presence of these substances in highly concentrated form only in the lumen of this convolution.

3. Special methods were devised to show convincingly the locus of this concentration, and the precise conditions under which this concentration occurs or may be induced are set forth.

4. Kidneys whose visible glomeruli are inactive, permitting the lumina of tubules connected with them to become filled with concentrated substance, are quickly washed free of it by reactivating these glomeruli.

5. The significance of the rapid release of fluid from the glomerulus is suggested and its effect on the relative patency of the lumen of the tubule is shown.

6. The rate of excretion by the tubule is relatively slow and uniform with or without continuous glomerular activity as shown by the careful inactivation of a single glomerulus in a highly functional kidney.

7. New methods are described for inactivating a given glomerulus as well as in part for studying segmentally, without sectioning, the tubule with which the inactivated glomerulus is connected.

8. Mechanical distention with fluid of the lumen of the tubule flattens the epithelium comparably with that observable in a diuretic kidney. The functional significance of this alone is doubtful.

9. Certain experiments on the frog involving ureteral injections indicate possibilities for a pyelovenous back-flow.

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